Prospective Trial of Arthroscopic Meniscectomy for Degenerative Meniscus Tears
NCT01931735

Study Protocol and Statistical Analysis Plan
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02a. Research Plan

Background and Significance

Guiding treatment of degenerative meniscus tears

Arthroscopic meniscectomy of the knee is among the most commonly performed orthopedic procedures in the United States and in the VA system. In 1999, it was estimated by the American Academy of Orthopedic Surgeons that 636,000 arthroscopic meniscectomies were performed annually in the United States (Praemer et al., 1999). Each procedure has been estimated to cost approximately $5000 (Moseley et al., 2002) leading to an estimated annual cost to the healthcare system of over three billion dollars.

Meniscus tears occur in a variety of settings and in patients with varied demographics, and various meniscus tear types have been described. Though there are certain tear types such as bucket-handle tears which are amenable to surgical repair, a large percentage of meniscus tears are not amenable to repair and, if treated surgically, are removed or debrided arthroscopically, a procedure known as arthroscopic partial meniscectomy.

One type of tear that is not repairable is the degenerative meniscus tear. This tear type usually has a horizontal component, or has a so-called "complex" pattern. It has been reported in multiple large studies that about 60% of meniscus tears treated arthroscopically are degenerative tears (Christoforakis et al, 2005, Metcalf et al., 2004). This tear type is associated with an older age group when compared with other tear types (Chirtoforakis et al., 2005). These findings closely reflect our practice at the Palo Alto VA hospital, and are likely to represent the general VA experience.

Degenerative meniscus tears are also associated with incipient osteoarthritis. A review of articular cartilage findings at the time of arthroscopy has revealed that complex and horizontal cleavage meniscal tears are highly associated with an increased incidence and severity of cartilage degeneration compared with other types of meniscal tears (Christoforakis et al, 2005). Long term follow-up of patients with degenerative meniscus tears who undergo partial meniscectomy reveals that they develop radiographic osteoarthritis at a higher rate than patients who have other tear types (Englund et al., 2003), likely because their knees already had some degree of arthritis at the time of surgery.

It is thus likely that about 60% of arthroscopic meniscectomy cases performed in this country, or roughly 380,000 cases annually, are performed on patients with degenerative meniscus tears and some degree of osteoarthritis. This is important because it has been clearly shown in a recent prospective, randomized, placebo controlled clinical trial in a VA patient population that arthroscopic debridement of the knee for the diagnosis of osteoarthritis is no better than placebo surgery (Moseley et al., 2002). Since nearly all patients undergoing arthroscopy and meniscectomy for degenerative meniscus tears also have some degree of chondromalacia or osteoarthritis, an important question that must be asked is: Does arthroscopic meniscectomy for a degenerative meniscus tear provide a benefit beyond the placebo effect?

Beyond the immediate effects that a patient undergoing meniscectomy for a degenerative tear may experience, another important concern regarding meniscectomy is the influence that meniscectomy has on the progression of osteoarthritis. Meniscectomy is known to have a number of biomechanical effects on the knee that can result in osteoarthritis. Meniscectomy dramatically alters patterns of stress in articular cartilage (Haemer et al., 2011, Baratz et al., 1986) and alters the position of the femur in relation to the tibia (Netravali et al., 2010). Meniscectomy is used in multiple animal models to generate osteoarthritis (Appleyard et al., 2003, Hashizume et al., 2010) Patients undergoing meniscectomy, and even partial meniscectomy, for meniscus tears are at elevated risk for developing knee osteoarthritis compared to matched controls (Englund et al., 2003). Yet in patients who have meniscus tears and end up having a meniscectomy it is unknown whether the meniscus tear itself was the reason for the elevated rate of late osteoarthritis, or whether the surgical procedure was the

cause. Given this information, another important question to be asked is: Does arthroscopic meniscectomy for a degenerative meniscus tear alter the kinematics of the knee and accelerate osteoarthritis development?

Answering these two questions, which correspond to the aims of the proposed work, would have an important effect on the treatment and rehabilitation of a very large group of veteran patients with degenerative meniscus tears and knee pain. Optimizing rehabilitation of these patients with treatments that are proven to be efficacious, and eliminating costly, ineffective, or harmful treatments is an important goal of this study. Answering these two questions can either justify the large expenditures of health care dollars presently used to surgically treat degenerative meniscus tears, or if the surgery is found to be ineffective or damaging, can identify a very important area for cost savings.

Understanding mechanisms of osteoarthritis progression

Osteoarthritis is the fourth most common health condition served by the Department of Veterans Affairs (Kazis et al., 1998), and is the leading cause of disability in the United States (CDC 2001). Across the general population, the prevalence of symptomatic knee arthritis in adults over age 45 was been estimated to be 17% (Jordan et al., 2007). Twenty three percent of Veterans over age 50 are diagnosed with osteoarthritis (Kazis et al., 1998). Clearly, there is an important need to understand the development and progression of osteoarthritis and to identify predictors of osteoarthritis progression. Such and understanding would help to guide innovative early therapies for osteoarthritis of the knee and to monitor the effectiveness of such treatments.

As a part of our effort to understand and monitor the progression of early osteoarthritis from a functional/biomechanical standpoint, and to understand the functional/biomechanical effects of meniscectomy that may lead to osteoarthritis, we ask in this study whether resection of an already damaged meniscal fragment leads to a kinematic change in the knee that has been associated with osteoarthritis progression in a number of different clinical settings - meniscectomy and ACL deficiency (Netravali et al., 2010, Andriacchi et al., 2006).

It may be that the degenerative meniscus is, by objective mechanical and functional measures, biomechanically incompetent to the point where it no longer serves any protective role to the articular cartilage or any stabilizing role to the joint. If this is the case, the kinematic changes that we have observed following arthroscopic meniscectomy (namely external rotation of the tibia in relation to the femur during the stance phase of gait) should be present prior to surgical removal of the torn meniscal fragment. If this is the case, it may be that partial meniscectomy itself (the removal of the torn fragment of meniscus) does not change the kinematic positioning of the tibia in relation to the femur, and is not actually the cause of post-meniscectomy osteoarthritis as has been implied in previous work (Englund et al., 2003). In this situation, the higher rate of osteoarthritis seen years after knee arthroscopy and partial meniscectomy may simply be due to the damage to the joint that led to the damaged meniscus. This is the issue we are trying to address with Aim 2 of our study. Assessing the biomechanical competence and function of the degenerative meniscus is central to the decision to remove the torn, but stable fragment.

Similarly, it has been suggested that the knee with a degenerative meniscus tear already has incipient osteoarthritis (Englund et al., 2003), even if osteoarthritis is not evident on plan radiographs. Recent work in the laboratory of Dr. Robinson (Wang et al., 2011) has revealed that there is an elevated level of activity in the complement cascade in the synovium of patients with early and end-stage osteoarthritis of the knee, with the most dramatic activation of the complement system observed in early knee OA. Using a mouse model of medial meniscal transection (Kamekura et al., 2005) work in Dr. Robinson's laboratory has been shown in this animal model of surgically-induced osteoarthritis that progression of osteoarthritis is dramatically slowed in genetic knockout mice that are missing enzymes involved in certain steps of the

complement cascade. Blocking of these critical steps in the complement cascade with antibodies has a similar effect.

Whether intervening on the complement system would slow the progression of mechanically/surgically induced osteoarthritis is not yet known in humans. In humans with advanced osteoarthritis undergoing total knee arthroplasty, elevated activity of the complement system has been established . Although the Robinson lab has demonstrated increase activation of the complement system in early OA (Fig. below; Wang et al., 2011), whether meniscal tears are associated with activation of the complement system and alteration of gene expression in the synovial lining has not been previously investigated. Establishing whether this is the case with joint fluid aspiration and a synovial biopsy of patients undergoing arthroscopic meniscectomy for a degenerative meniscus tear, since they are believed to be patients with early osteoarthritis, will be a major step in further understanding the role of complement in the progression of osteoarthritis and potentially developing pharmacologic intervention strategies to slow osteoarthritis progression. This the focus of an exploratory aim of the proposed work.

Preliminary Studies and Current Status of the Field

Prospective Study on Arthroscopic Meniscectomy outcomes at the VA Palo Alto

We have performed a preliminary study on patients at the Palo Alto VA hospital undergoing arthroscopic meniscectomy (Mirza et al., 2011). In this study, we prospectively followed patients presenting to the orthopedic clinic with meniscus tears and who underwent arthroscopic meniscectomy. 78 subjects with a mean age of 51 years scheduled for knee arthroscopy for meniscal tears were enrolled. 45 subjects were available for final review at one year. History of acute injury within 6 months of surgery, Kellgren-Lawrence arthritis grade, and MRI appearance of meniscus tear (degenerative or not) was recorded for each patient. Pre- and one year postoperative WOMAC and WOMET were used to evaluate surgical outcome with statistical analysis done by ANOVA.

The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) (Bellamy et al., 1988) and the more recent Western Ontario Meniscal Evaluation Tool (WOMET) (Kirkley et al., 2007) are self-administered validated outcome tools for osteoarthritis of the knee and meniscal tears of the knee.

The psychometric properties of the WOMAC have been extensively studied in a number of publications. The original publication by Bellemy (Bellemy et al., 1988) found a high level of responsiveness, Chronbach's alpha greater than 0.85, acceptable validity testing against multiple other scales including the Lequesne, Bradburn, and Doyle, and superior efficiency over other measures.

The psychometric properties of the WOMET in a population of patients with degenerative meniscal tears were recently published (Sihvonen et al., 2012). Test-retest reliability 95% limits of agreement were 20.1 and -20.11 as a percentage of normal score. Cronbach alpha score for total WOMAC was 0.91 and was greater than 0.7 for all domains. Floor and ceiling effects were less than 30%. The WOMET was significantly correlated to the Lysholm knee score ($r=0.558$, $p<0.001$) and the 15-D scale ($r=0.311$, $p=0.002$). Construct validity and responsiveness were also established.

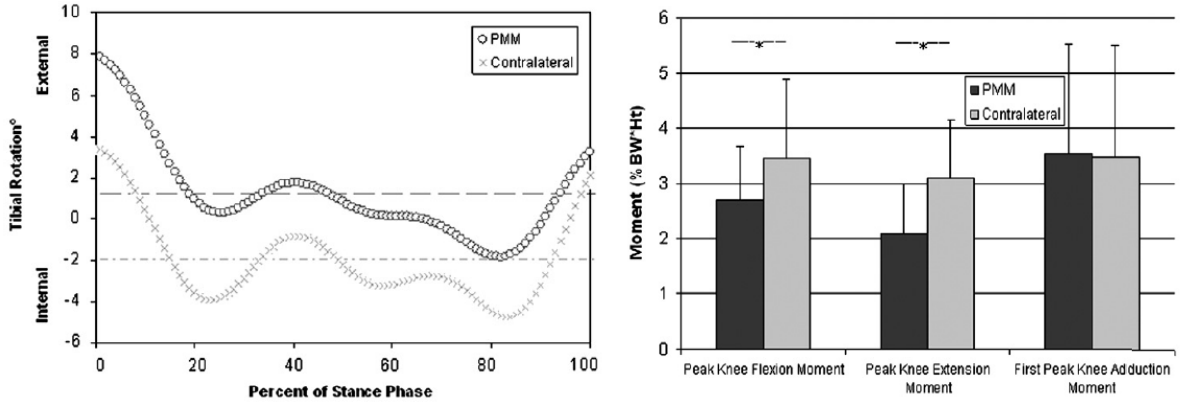
Preoperative baseline WOMET and WOMAC scores were compared to postoperative values at one year. Subjects were also defined according to age (greater than 50 or younger), BMI, OA grade (K-L classification), meniscal tear type (complex or simple), smoking history (yes/no), psychiatric diagnosis (yes/no), and PTSD diagnosis (yes/no). Two orthopaedic surgeons evaluated the xrays and MRI films independently with discrepancy resolved through consensus. Other variables were identified through detailed chart review of diagnoses and parameters. Forty-five subjects completed one-year followup and were available for analysis. WOMAC and WOMET scores improved significantly in the entire cohort at one year ($p<0.0001$). Patients with

OA grade 0-1 had greater improvements than those with grade 2-3. Mean differences in WOMET and WOMAC scores overall (95% CI, p-value) in 45 subjects at 1 year were 354 (224-483; p<0.0001) and 561 (407-714, p<0.0001); respectively. Subgroup analysis based on lower OA grade (0 and 1) revealed mean differences in WOMET and WOMAC scores of 456 (290-621, p<0.0001) and 656 (466-845, p<0.0001) at 1 year; respectively. Chronicity of symptoms and tear type had no effect on outcomes. Other variables such as age, BMI, type of meniscus tear, smoking history, and psychiatric diagnosis had no significant impact on the results.

This preliminary study gave us experience recruiting and following arthroscopic meniscectomy patients and experience with the WOMAC and WOMET, the primary outcome measures for the current study. We defined mean and standard deviation scores in our meniscectomy patient population that allow us to perform a power analysis for our proposed study. Furthermore, we defined a covariate, radiographic severity of osteoarthritis, that appears to have an effect on outcomes. This study lays a good foundation for the current study that is being proposed. It strongly suggests the need for a prospective study on arthroscopic meniscectomy for degenerative tears that has a control group, as the improvement in the arthroscopic meniscectomy patient population we observed in our pilot study is comparable in magnitude to the improvements seen after placebo surgery (Moseley et al., 2002) and physical therapy (Kirkley et al., 2008) interventions for knee osteoarthritis.

Effects of Meniscectomy on Gait and Functional Measures

We have recently completed a study with gait analysis on patients who have had arthroscopic medial meniscectomy (Netravali et al., 2010). The menisci are known to influence the transverse plane movements (anterior-posterior (AP) translation and internal-external (IE) rotation of the knee during walking. In that study we sought to determine the influence of partial medial meniscectomy on the kinematics and kinetics of the knee during the stance phase of gait by testing the differences in AP translation and IE rotation, knee flexion range of movement, peak flexion and extension moments, and adduction moments between the meniscectomized knees and the healthy contralateral knees in ten patient ranging from 45 to 79 years of age.



Figures 1 and 2: Kinematic and kinetic differences between knees with and without posterior medial meniscectomy (PMM) (Figures from Netravali et al., J Biomechanics 2010)

We found that the primary kinematic difference was a significantly greater external rotation of the tibia through the stance phase of gait with eight of ten subjects demonstrating this pattern. Limbs that had undergone posterior medial meniscectomy also had lower peak flexion and extension moments compared to the normal limbs. We interpreted this result as resulting in changes of tibio-femoral contact during walking, which could be a mechanism for initiating the process of post-meniscectomy osteoarthritis.

This study shows that we have the ability to identify changes in knee kinematics and kinetics after meniscectomy using our current gait analysis techniques. A key piece of information that is missing, however, is the kinematics of the knee with the meniscus tear that has not been debrided. Though we have seen these kinematic changes in knees during gait after meniscectomy, it is not clear whether these changes were actually caused by the meniscectomy or by the injury/meniscus tear itself. This is an important distinction as it begins to probe the etiology of post meniscectomy osteoarthritis. If we find that these kinematic changes in knees during gait are present only after meniscectomy, we would have reason to believe that post-meniscectomy osteoarthritis is a result of the surgical intervention itself (partial meniscectomy) rather than just due to the meniscus tear.

Relation between knee walking kinematics and spatial cartilage thickness distribution in the knee

We have done previous work relating the kinematics of walking on the spatial distribution of articular cartilage thickness in the knee (Koo et al., 2011). Gait data and knee MR images were obtained from 17 healthy volunteers (age 33.2 ± 9.8 years). Cartilage thickness maps were created for the femoral and tibial cartilage. Locations of thickest cartilage in the medial and lateral compartments in the femur and tibia were identified using a numerical method. The flexion-extension (FE) angle associated with the cartilage contact regions on the femur, and the anterior-posterior (AP) translation and internal-external (IE) rotation associated with the cartilage contact regions on the tibia at the heel strike of walking were tested for correlation with the locations of thickest cartilage. The locations of the thickest cartilage had relatively large variation (SD, 8.9°) and was significantly associated with the FE angle at heel strike only in the medial femoral condyle ($R(2)=0.41$, $p<0.01$).

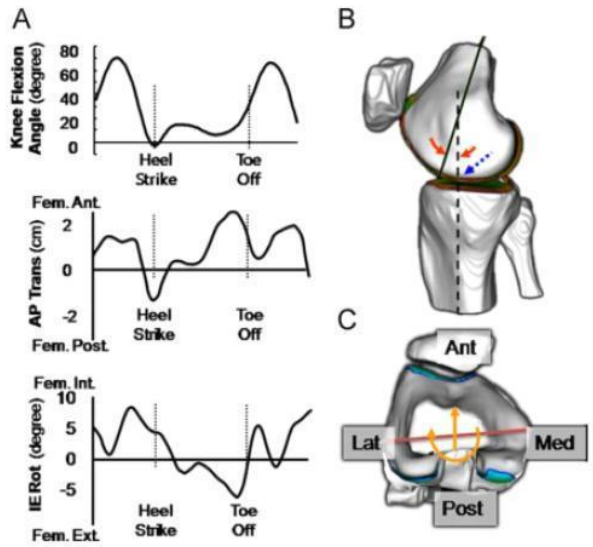


Fig. 3. (A) Primary (FE) and secondary (AP and IE) kinematics in the knee during walking, (B) femoral contact location (dotted arrow) predicted from FE angle (solid arrow), and (C) tibial contact location (solid line) predicted from AP translation and IE rotation.

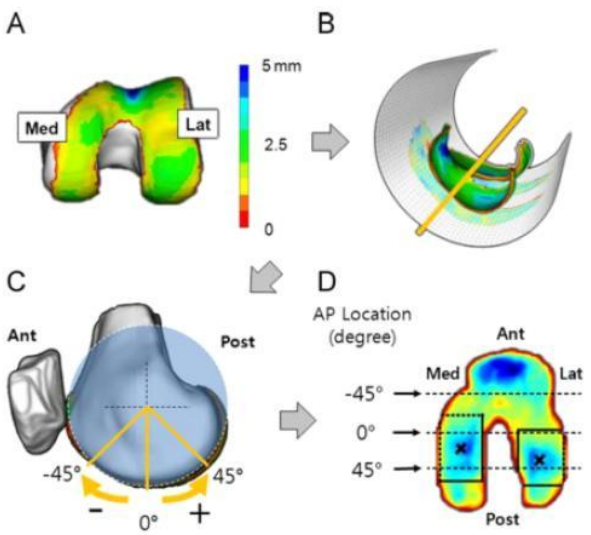


Fig. 4. (A) Femoral cartilage thickness map, (B) a fitted cylinder to project the thickness map, (C) rotational AP location represented as degree relative to the long axis of the femur, and (D) rectangular search region on each condyle to find the location of thickest cartilage in the flattened cartilage thickness map.

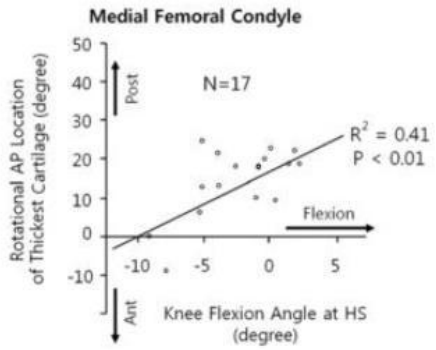


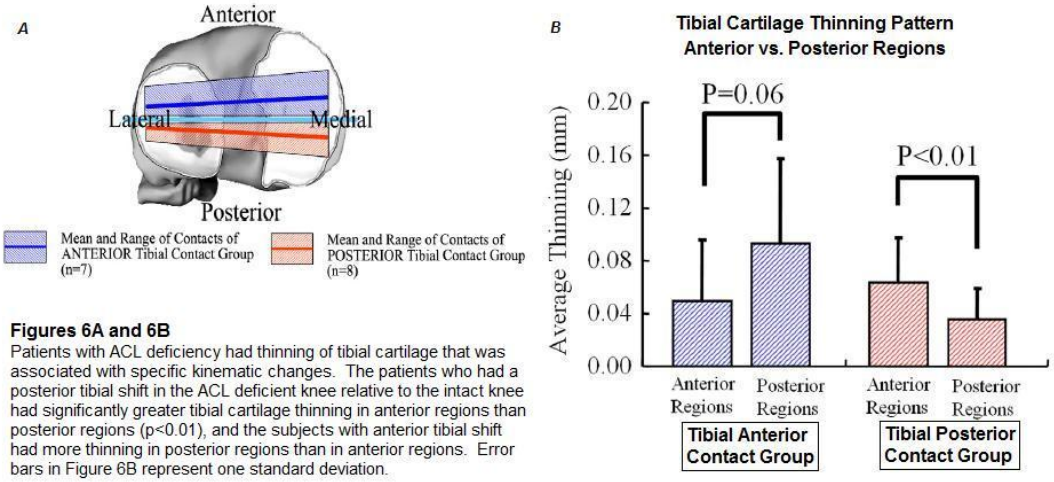
Fig. 5. Knee flexion angle at heel strike was significantly associated the rotational AP location of the thickest cartilage only in the medial femoral condyle.

(Figs 3-5 from Koo et al., 2011)

The natural knee kinematics and contact surface shapes seem to affect the functional adaptation of knee articular cartilage morphology. The sensitivity of cartilage morphology to kinematics at the knee during walking suggests that regional cartilage thickness variations are influenced by both loading and the number of loading cycles. Thus walking is an important consideration in the analysis of the morphological variations of articular cartilage, since it is the dominant cyclic activity of daily living. The sensitivity of cartilage morphology to gait kinematics is also important in understanding the etiology and pathomechanics of osteoarthritis.

Gait mechanics influence healthy cartilage morphology and osteoarthritis of the knee

In another study, the response of healthy and diseased cartilage of the knee to the mechanics of walking was examined, with the goal of providing insight into the relationship between the kinematics and kinetics of the knee during walking and the maintenance of cartilage health Andriacchi et al. 2009). The combination of information from three-dimensional thickness models of cartilage derived from magnetic resonance imaging and the analysis of the interaction between load at the knee and kinematic changes during walking associated with loss of the anterior cruciate ligament demonstrated the importance of considering walking mechanics as an important factor in the initiation and progression of osteoarthritis. In particular, this material suggested that knee cartilage becomes conditioned to loading and to the large number of repetitive cycles of loading that occur during walking and that healthy cartilage homeostasis is maintained as long as there are no changes to the normal patterns of locomotion, the structure of the knee joint, or cartilage biology. Thus, there is the potential for a degenerative pathway to be initiated when a condition such as anterior cruciate ligament injury causes the repetitive loading during walking to shift to a new location.



Figures 6A and 6B
Patients with ACL deficiency had thinning of tibial cartilage that was associated with specific kinematic changes. The patients who had a posterior tibial shift in the ACL deficient knee relative to the intact knee had significantly greater tibial cartilage thinning in anterior regions than posterior regions ($p<0.01$), and the subjects with anterior tibial shift had more thinning in posterior regions than in anterior regions. Error bars in Figure 6B represent one standard deviation.

(From Andriacchi et al., 2009)

The sensitivity of cartilage to the kinematic changes is illustrated with the anterior cruciate ligament-deficient knee and the regional variations in cartilage morphology (Figure 6). The material presented here supports the conclusion that individual variations in the range of loading and kinematics at the knee during walking can have a profound influence on the initiation and progression of osteoarthritis of the knee. In this study, we will perform a similar analysis, but one that looks at the kinematic shifts associated with meniscus tearing or debridement. Figure 7 shows the conceptual model of osteoarthritis that forms the basis for this investigation.

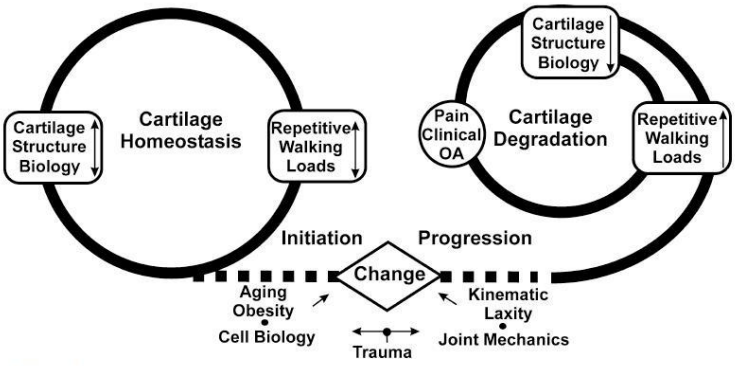


Figure 7
Healthy cartilage homeostaisis is maintained by magnitude of repetitive cyclic loads during walking, and cartilage is thicker in regions with higher loads during walking. The initiation of osteoarthirtis is associated with a change (due to injury, increased laxity, neuromuscular changes, aging, obesity) in the normal balance between the mechanics of walking and the cartilage biology or structure. Once cartilage starts to degrade, it responds negatively to load and the rate of progression of osteoarthritis increases with loading.

(From Andriacchi et al., 2009)

Biomechanical benefit of retaining meniscal tissue in a horizontal meniscus tear

We performed and published a study looking at the horizontal meniscus tear pattern, which is the most common pattern of meniscus tear in the degenerative meniscus (Haemer et al., 2007). In this cadaveric study, we evaluated the contact stresses on the tibial articular surface when the meniscus is cut horizontally, simulating a horizontal meniscus tear. We found that a cut in the meniscus itself does not change the contact area or contact stress substantially on the tibial articular surface. Sparing one leaf was beneficial compared to resecting both leaves because resection of the second leaf reduced contact area an additional 15%. Similarly, mean pressure was increased 24% for single-leaf resection and an additional 27% for double-leaf resection. Peak pressure showed no differences with single- and double-leaf resections.

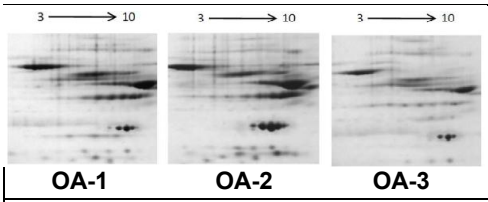
Table 1: Effects of tear and partial removal on tibial contact area and pressures

Meniscus Condition	Contact Area (mm^2)	Mean Pressure (MPa)	Peak Pressure (MPa)
	Mean (Standard Error)		
Intact	105 (5)	4.3 (0.3)	9.4 (0.4)
Horizontal tear	98 (5)	4.1 (0.3)	9.1 (0.4)
Single-leaf removal	64 (5)	5.3 (0.3)	10.3 (0.4)
Double-leaf removal	48 (5)	6.4 (0.3)	10.6 (0.4)

(From Haemer et al., CORR 2007)

This study shows us that retention of all meniscal tissue in the setting of a stable horizontal meniscus tear may be beneficial to the articular cartilage of the joint from the perspective of contact area and contact stress. If we find that, from a short term clinical standpoint, there is no symptomatic benefit to resecting any part of a stable horizontal meniscus tear, there is reason to believe from this study that the retained tissue will protect cartilage from reduced contact area and excessive contact stress.

Proteomic survey of OA synovial fluid. The Robinson lab subjected OA synovial fluid samples to 2-dimensional gel electrophoresis followed by mass spectrometric analysis (the Robinson Laboratory runs a mass spectrometry core for the VA Palo Alto). 183 distinct proteins were identified, and the DAVID bioinformatic algorithm (http://david.abcc.ncifcrf.gov/) was used to determine the pathways represented by the identified proteins along with the Bonferroni-corrected *P* value for each pathway (Fig. 8 below).



Mass spectrometry: 193 proteins identified

Pathways identified	# proteins	*P* value
"Complement & coagulation"	24	3.8×10^{-21}
"ECM-receptor interaction"	17	4×10^{-10}
"Focal adhesion"	24	4×10^{-10}
"Toll-like receptor signaling"	11	0.002
"Cytokine-cytokine receptor"	16	0.01

Fig. 8. Proteomic survey of high abundance proteins in OA synovial fluid. Ten OA synovial fluids were separated by 2-dimensional gel electrophoresis (representative gel images presented), and protein spots and other gel regions were isolated, in-gel trypsin digestion performed, and an Agilent XCT ion trap mass spectrometer used to identify proteins. 193 distinct proteins were identified, and the DAVID website version 6.7 applied to identify the pathways represented. The pathways identified, number of distinct proteins per pathway identified, and the Bonferroni-corrected *P* value are listed.

Complement components are aberrantly expressed and activated in OA joints. Complement proteins identified in our proteomic survey of OA synovial fluid are listed in Figure 9 below. Components of the classical (C1s & C4a) and alternative (factor B) pathways, the C3 and C5 convertases, and the C5, C7, and C9 components of MAC were aberrantly expressed in OA (Wang et al, 2011).

Proteins Identified:	Swiss-Prot Reference:
Complement C1s subcomponent	P09871
Complement C3	P01024
Complement C4-A (Acidic complement C4)	P0C0L4
C4b-binding protein alpha chain (C4bp)	P04003
Complement C5	P01031
Complement C7	P10643
Complement C9	P02748
Complement factor B	P00751
Complement factor H	P08603
Complement factor I	P05156
Clusterin	P10909
SERPING1 (C1 Inhibitor)	P05155

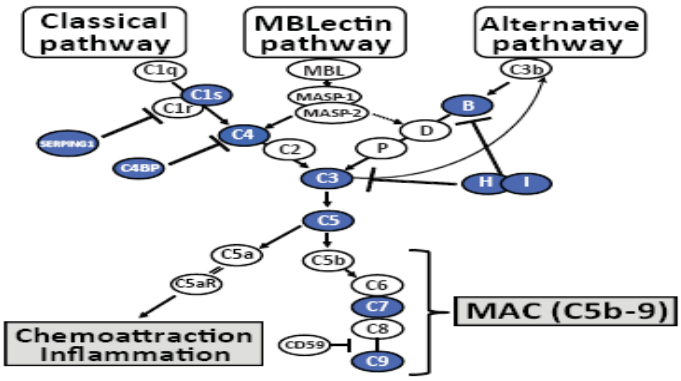


Fig. 9. Complement components are aberrantly expressed and activated in OA joints. Complement proteins identified by 2-dimensional gel electrophoresis and mass spectrometric analysis as expressed in OA (*n* = 10) compared to healthy (*n* = 10) synovial fluids.In the schematic of the complement cascade, blue-filled circles denote the complement effectors and inhibitors identified as expressed in OA.

Increased complement proteins in OA synovial fluid. We used ELISA to verify the presence of complement in synovial fluid in OA, and found that C3a and MAC (C5b-9) levels are increased in OA compared to healthy synovial fluid, early in the course of OA (Fig. 10 below; Wang et al, 2011). Elevated levels of MAC in OA synovial fluids have been reported previously.

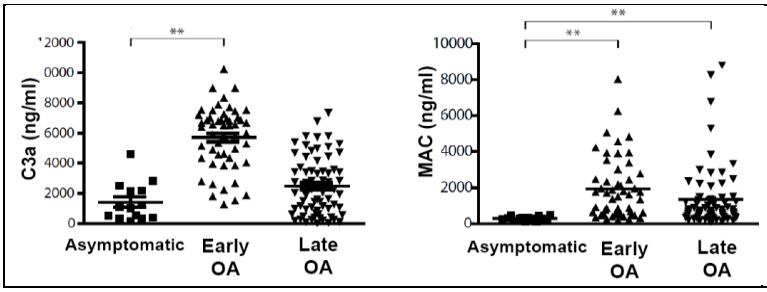


Fig. 10. Complement is aberrantly hyperactivated in human OA. ELISA quantification of C3a and of the soluble form of MAC in synovial fluids from asymptomatic healthy individuals (*n* =14) and from patients with early-stage OA (*n* = 52) or end-stage OA (*n* = 69). ***P* ≤ 0.01 by one-way ANOVA and Dunnett's post-hoc test.

MAC deposition in OA cartilage and synovium. We performed immunohistochemistry on cartilage and synovium from patients with end-stage OA by using an antibody specific for MAC (C5b-9). Staining with anti-C5b-9 demonstrates the presence of MAC on and around chondrocytes in the OA cartilage, and on and around synoviocytes in the OA synovium (Fig. 11 below). Our results are consistent with previous reports demonstrating C3 on articular surfaces and MAC on and around chondrocytes[19] in OA cartilage (Wang et al, 2011).

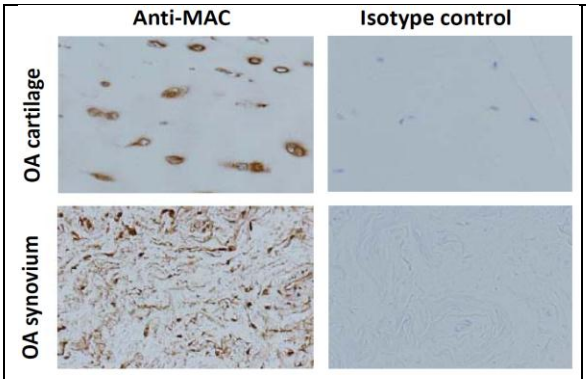


Fig. 11. Immunohistochemistry demonstrates deposition of membrane attack complexes (MAC, C5b-9) on and around chondrocytes in OA cartilage and synoviocytes in OA synovium. OA cartilage and synovium collected by the Stanford Arthritis Center at the time of arthroplasty was obtained and stained with an anti-MAC (C5b-9) antibody. Samples were fixed, paraffin-embedded, and stained with antibody specific for C5b-9 or an isotype-matched antibody, and HRP-conjugated secondary antibodies were used to detect primary antibody reactivity. Similar staining patterns were observed in 4 independent samples; representative images are presented.

Activation of the complement system by OA cartilage. *In vitro* complement assays were performed as described[22]. Pulverized OA cartilage was added to human serum, and ELISAs were performed to quantify MAC production as a measure of complement activation. Human OA cartilage activated MAC production over that observed with OA synovium or PBS (Fig. 12 below). We further demonstrate that the cartilage component fibromodulin is increased in OA synovial fluid and can directly activate the complement system to result in the formation of C5b-9 (Fig. 13).

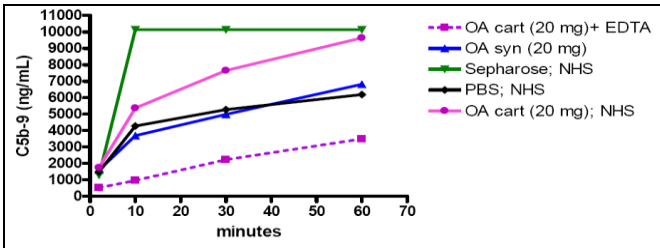
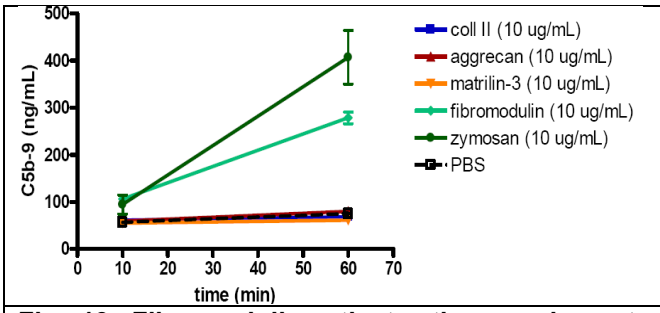


Fig. 12. OA cartilage induces production of MAC (C5b-9). Pulverized OA cartilage (OA cart), OA synovium (OA syn), PBS (negative control), or sepharose (positive control) was added to normal human serum (NHS), and production of MAC quantified by ELISA at various time points. OA cartilage, but not OA synovium, activated the complement system, resulting in production of MAC.

Increased complement-effector, and decreased complement-inhibitor, gene expression in OA synovium. To determine whether the increase in levels of complement effector proteins in OA synovial fluid (Fig. 13 below) is due to aberrant production of these factors by joint tissues, we performed hierarchical clustering of publicly available gene-expression datasets. Our analysis revealed two major clusters, one containing the expression profiles from OA patients and another containing the profiles from healthy individuals (Fig. 14 below; Wang et al, 2011). Surprisingly, we found a striking induction of transcripts encoding complement components of the alternative pathway (C3, C7, C4A, factor I, factor B, C9, C5) and a concomitant suppression of inhibitors of the alternative pathway (factor H, C1 inhibitor, C4-Acidic) in OA synovial tissue in both early and end-stage OA synovial linings. The converse was observed in healthy synovium. These results suggest that complement-encoding genes are dysregulated in early and end-stage OA synovium.



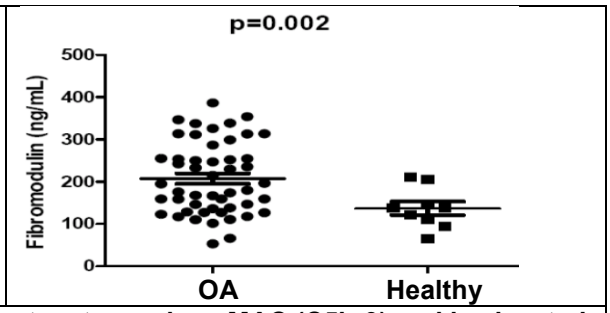


Fig. 13. Fibromodulin activates the complement system to produce MAC (C5b-9) and is elevated in OA synovial fluid. (a) Fibromodulin was added to 10% human serum and production of MAC (C5b-9) was measured by ELISA. $P \leq 0.01$ by one-way ANOVA with Dunnett's post-hoc test comparing PBS with fibromodulin, matrilin-3, aggrecan, or type II collagen. Zymosan was used as a positive control. (**b**) ELISA quantification of fibromodulin levels in synovial fluids from OA patients ($n = 50$) and healthy individuals ($n = 9$). $^{**}P \leq 0.01$ by Student's *t*-test.

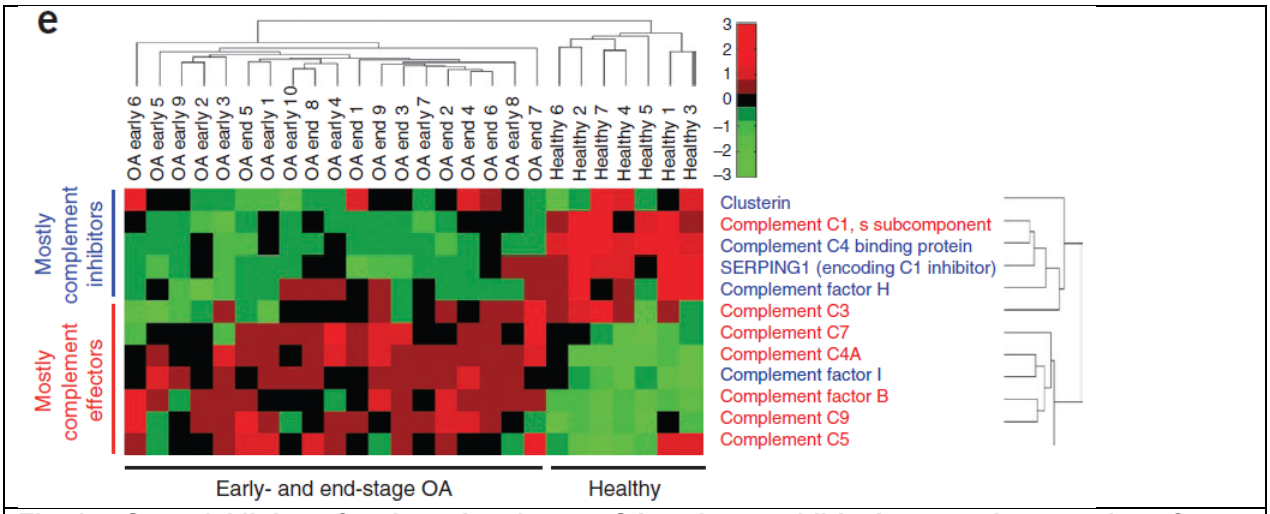


Fig. 14. Synovial lining of early and end-stage OA patients exhibits increased expression of genes encoding complement-effector proteins, and decreased expression of genes encoding complement-inhibitory proteins. Cluster analysis of gene-expression profilings in microarray datasets from synovial membrane derived from early and end-stage OA patients were experimentally determined using Affymetrix Human U133 Plus 2.0 chipsand healthy controls (accession # GSE12021) were downloaded from the NCBI's Gene Expression Omnibus (GEO). For transcriptional profiling, end-stage knee OA synovial membranes were obtained from patients undergoing knee joint replacement and early-stage knee OA synovial membranes were obtained from patients undergoing arthroscopy for meniscal tears with cartilage degeneration but no full-thickness cartilage loss. The results for the complement-related proteins identified by mass spectrometry (Fig. above) were extracted, subjected to hierarchical clustering, and displayed as a heatmap. Red = increased RNA expression; black = no difference; green = decreased RNA expression.

Increased cytokines in OA sera and synovial fluids. To follow up on our mass-spectrometry results, which included identification of proteins that characterize "Immunologic disorder(s)" (Fig. 15 below), the Robinson lab used optimized protocols (Hueber et al, 2008) to perform Luminex analysis of the cytokines present in synovial fluid and serum samples derived from patients with OA or rheumatoid arthritis (RA) or from healthy individuals. We show that, though low, levels of cytokines are significantly higher in sera from OA patients compared to healthy individuals (false discovery rate (FDR) < 0.05)(Fig.13 below, right panel). We also profiled cytokine levels in healthy sera, OA sera, OA synovial fluids, RA sera, and RA synovial fluids (FDR < 0.01) (Fig.15 below, left panel). Cytokine levels are slightly increased in OA sera compared to healthy sera, significantly increased in OA synovial fluids compared to healthy synovial fluids, and highest in RA sera and synovial fluids (Fig. 15 below, left panel).

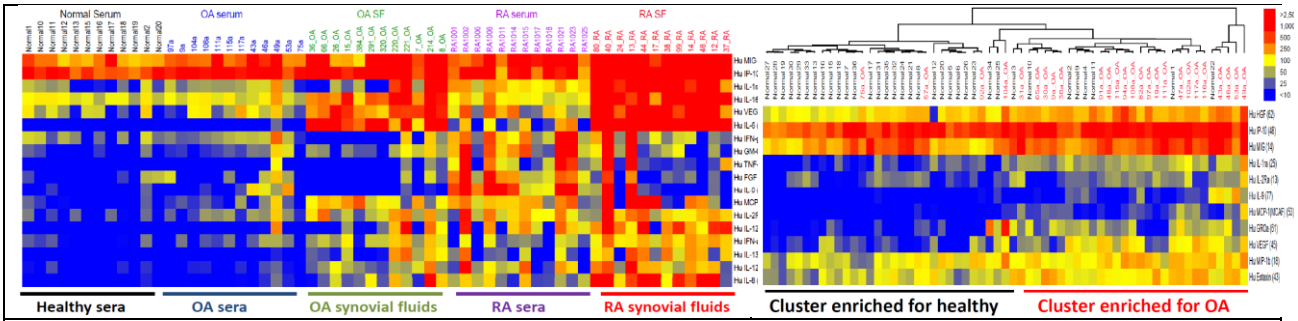


Fig. 15. Increased cytokines in OA serum and synovial fluid. The Luminex System was used for profiling of cytokines in sera and synovial fluid samples provided by the Stanford Arthritis Center, according to protocols optimized by the Robinson lab (Hueber et al, 2007). SAM was used to identify

statistical differences between levels of cytokines in healthy (normal) sera, OA sera, OA synovial fluid, RA sera, and RA synovial fluid, and statistically significant results are presented in the heatmap on the left (FDR < 0.01). In the right panel, SAM identified cytokines increased in OA sera as compared to healthy sera (FDR < 0.05), the results subject to unsupervised clustering, and the output displayed as a heatmap.

Imaging Background

Joint Morphology in OA with MRI. Magnetic resonance imaging offers excellent assessment of joint structure in OA {Conaghan, 2006}. Although three-dimensional MRI can provide accurate quantification of cartilage thickness and volume, these change slowly in OA {Eckstein, 2008}. Recent studies have shown focal cartilage thinning in a group of OA subjects in the weight-bearing regions in as little as one year {Wirth, 2010}. Other important joint structures such as the ligaments, menisci, synovium, and bone marrow that are active in the OA process have received far less attention than cartilage {Conaghan, 2006;Conaghan, 2004}. Conventional MRI of these structures is able to show internal derangements such as meniscal tears, which is a late finding and irreversible. However, early degenerative changes in fibrocartilage tissue such as ligaments and menisci are not routinely measured with conventional MRI due to the short T_2 relaxation times of these tissues. Short echo time or uTE imaging improves imaging in these tissues {Gatehouse, 2004} but requires specialized software and hardware.

Structural information from conventional intermediate-weighted 2D fast-spin-echo {Boileau, 2008} and 3D double-echo steady state (DESS) MRI can be summarized using semi-quantitative scoring systems such as BLOKS or WORMS {Hunter, 2008;Peterfy, 2004;Felson, 2010}. The changes seen in OA pathology detected on routine MRI include joint effusion, synovitis, bone marrow lesions, and cartilage matrix. These are useful to document the slow progression of OA but are insensitive to short-term changes in cartilage that may be reversible. Long-term assessment of regional cartilage thickness, as well as additional assessment of cartilage, menisci, and ligaments can be performed using DESS imaging {Eckstein, 2006;Guermazi, 2009}, the current method used in the OAI. Joint changes such as cartilage volume and regional thickness are assessed using post-processing segmentation. Regional cartilage thickness has been shown to change a measureable amount using MRI in 18 months from the OAI data {Wirth, 2010}.

Joint Physiology in OA with MRI. Recent advances in MRI contrast mechanisms have the potential to probe the extracellular matrix of cartilage. Several promising techniques that show early matrix change in OA include T_2 mapping, $T_{1\rho}$ mapping, dGEMRIC, and sodium imaging {Burstein, 2006}. An exciting new method of detection of glycosaminoglycan (GAG) by chemical exchange saturation transfer (gagCEST) has only recently been described {Ling, 2008}. All of these methods can be difficult to use in multi-center trials, which has limited their application in studies of interventions in OA. Comparisons between these methods have also been difficult, owing to a lack of compatible software and hardware at any one institution.

Measurement of the T_2 relaxation time has been shown to be a reflection of the organization of the collagen network within the cartilage {Mosher, 2004}. Increased T_2 relaxation time is a marker for matrix breakdown, which may occur before cartilage volume or thickness loss occurs. T_2 mapping is sensitive to magic angle effects and the hydration state of the cartilage. This makes T_2 mapping less specific for collagen content and more of a reflection of overall cartilage structure. T_2 mapping is usually done with a two-dimensional fast spin-echo approach that can take at least 10 minutes to cover an entire knee. Two-dimensional imaging in this case is a significant limitation that results in errors in quantification due to slice gaps and partial volume effects.

Several promising methods have been developed to assess cartilage GAG. Measurement of the relaxation of the spins in cartilage in the presence of a radio-frequency field

or $T_{1\rho}$ imaging has been shown to be useful in detecting changes to the extracellular cartilage matrix {Borthakur, 2006}. Although $T_{1\rho}$ is sensitive to matrix changes in OA, there is some controversy as to the specificity of $T_{1\rho}$ for changes to glycosaminoglycans {Menezes, 2004}.

Delayed gadolinium enhanced MRI of cartilage (dGEMRIC) is a well-established MRI method to measure GAG content in cartilage {Burstein, 2000}. dGEMRIC has shown high accuracy and promise in the research setting, but requires intravenous injection of double-dose gadolinium contrast and a significant delay before image acquisition. dGEMRIC has been used to demonstrate that GAG loss in cartilage is reversible with an exercise intervention {Roos, 2005}. Use of dGEMRIC is also limited by concerns about high-dose administration of gadolinium and nephrogenic sclerosing fibrosis, a rare but serious connective tissue disorder {Weinreb, 2009}.

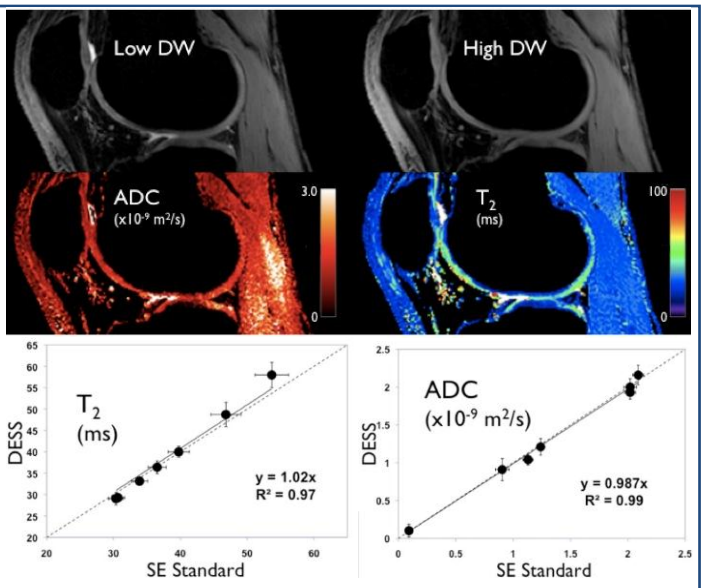


Figure 16: *Using qDESS acquisitions in 8 minutes, sum images (top) from acquisitions with low and high diffusion weighting can be used for segmentation. Further processing provides Apparent Diffusion Coefficient (ADC) and T_2 maps (middle). Controlled tests in phantoms show high accuracy of qDESS T_2 and ADC values compared with spin echo (SE) methods (bottom).*

Direct imaging of sodium with MRI provides a non-invasive assessment of cartilage GAG content. Sodium is the positive ion associated with the negatively charged GAG side chains in cartilage, so sodium provides a direct measure of GAG in cartilage {Borthakur, 2006}. MR imaging of sodium has traditionally been limited by low concentration, low gyromagnetic ratio, and short T_2 relaxation times. However, recent advances in MR imaging using non-Cartesian acquisition strategies have enabled acquisition of volumetric sodium data in cartilage at clinical field strengths *in vivo* {Staroswiecki, 2010}.

Diffusion MRI may also be sensitive to cartilage GAG content. A recent study showed excellent correlation between ADC and GAG content in artificially depleted cartilage specimens {Raya, 2011}. Diffusion-weighted MRI of cartilage has been attempted *in vivo* with limited success {Azuma, 2009;Miller, 2004}.

DESS is a rapid 3D method that produces two images: one with high signal and one with more T_2 contrast. {Hardy, 1996}. Normally these images are simply added, giving sufficient contrast for cartilage segmentation {Eckstein, 2006}, as has been used extensively in the OAI. More recently, it was described that the signal ratio in DESS may be used to provide an approximation to a T_2 map, with much higher spatial resolution and shorter scan times than conventional T_2 mapping {Welsch, 2009}. We have extended this further by incorporating fat suppression and varying the flip angle and spoiler gradient, to modulate the diffusion sensitivity {Buxton, 1993}. This is based on our pervious work in cartilage diffusion imaging. Overall, this new approach offers an efficient, high-resolution single-acquisition of 3D images for segmentation, T_2 maps, and ADC measurement.

Figure 16 summarizes our validation of both T_2 and ADC measurement using quantitative DESS (qDESS) {Staroswiecki, in press}. In 5 healthy subjects, we acquired qDESS images with different diffusion weighting, and fit a signal model to generate T_2 and ADC maps. Both were compared with spin-echo measurements of T_2 and ADC, with excellent agreement. Additionally,

we validated accuracy of qDESS in phantoms of varying T_2 and diffusivity. The agreement with standard spin-echo methods is excellent. Although the validation of diffusion measurement is preliminary, substantial work has already been performed to validate DESS for segmentation {Wirth, 2009} and semi-quantitative scoring {Roemer, 2009}. qDESS is particularly promising for cartilage assessment, as it avoids the blurring and signal loss of spin echo methods as well as the image distortion in most diffusion-weighted imaging sequences.

Imaging of muscle cross sectional area: Muscle cross sectional area measurement can provide additional information regarding the functional consequences of meniscectomy. We will image the thigh of patients at enrollment, and at two-year follow up to determine if patients undergoing meniscectomy vs. lavage have greater changes in muscle cross sectional area. Images will be obtained using a rapid axial thigh scan with IDEAL fat/water separation using a 3D spoiled gradient echo sequence. The data will have 1 mm in-plane resolution and 5 mm slice thickness. Acquisition time will be approximately 2 minutes. Muscle cross sectional area will then be determined as previously described {Walton 1997}.

Research Design and Methods

In this study we propose a prospective, randomized, blinded, and controlled clinical trial of arthroscopic meniscectomy in patients between the ages of 45 and 70 with degenerative meniscus tears.

Patient Selection:

Subjects will be recruited from the orthopedic clinic at the VA Palo Alto Healthcare System. Eligible subjects will meet the following inclusion criteria:

1. Age between 45 and 70 years
2. Degenerative meniscus tear as seen by a standard clinical MRI scan
3. A complaint of “mechanical symptoms” which would include popping, catching, or locking of the knee
4. Persistent symptoms for a minimum of three months despite conservative management including anti-inflammatory medications and physical therapy
5. Patient is enrolled for arthroscopic meniscectomy
6. The subject agrees to study follow-up schedule and signs informed consent.

The following exclusion criteria will be enforced:

1. Radiographic osteoarthritis of the knee in any compartment that is greater than KL grade 2
2. Prior knee arthroscopy within two years of study enrollment
3. Unable to have an MRI scan
4. Does not agree to participate

General Study Plan:

Patients who satisfy the inclusion and exclusion criteria and are scheduled for arthroscopic meniscectomy will be approached during their preoperative visit to participate in the study. They will be enrolled when they give informed consent.

Preoperative study related evaluation:

Prior to undergoing surgery, the patient will have the following evaluations:

1. The patient will fill out the WOMET (Kirkley et al., 2007) and WOMAC (Bellamy et al., 1988) surveys.
2. The patient will undergo a gait analysis
3. The patient will have MRI of the knee and thigh.
4. The patient will have standing radiographs of the affected knee.

Evaluation at the time of surgery:

At the time of surgery, the patient will be randomized to treatment with either an arthroscopic partial meniscectomy or arthroscopic lavage. Randomization will be done by opening a sealed envelope in the operating room prior to the procedure. To ensure balanced numbers in the two surgical conditions, especially at the time of the interim look, randomization will be done by permuted blocks of size 10, thereby ensuring 5 patients in each group at every increment of 10 enrollees.

Upon initiation of the surgery, a sample of synovial tissue and synovial fluid will be obtained for biochemical analysis. During the operation, the status of the knee joint will be documented. This will include classifying the degree of chondromalacia according to the Outerbridge classification in all three compartments of the knee, and documenting the geometry and pattern of meniscus tear type. Video and photographs will be taken to assist with this documentation and will be stored in a secure manner.

During the operation, the meniscus will be carefully probed. If a large unstable flap of tissue can be displaced into the articulation (such as a bucket handle tear) this torn fragment will be debrided regardless of the result of randomization. This will be done as it is widely believed that it is inappropriate to leave such an unstable tear untreated. Any other tear types that do not result in an unstable flap that can be displaced into the articulation will be either retained or debrided, depending on the result of randomization.

<u>All postoperative contact from this point forward will be made by study personnel who were not involved in the surgery and who do not have knowledge of the randomization result.</u>

At 2 weeks and 6 weeks:

The patient will have regularly scheduled clinic visits at 2 and 6 weeks that are not a part of the study but are just a part of the routine postoperative care of knee arthroscopy patients. Postoperative care as deemed necessary by the treating physician will be provided and could include pain and/or anti-inflammatory medications. Physical therapy is rarely needed. All provided treatments will be recorded in the chart.

Evaluation at 3 months:

1. The patient will fill out WOMET and WOMAC surveys.
2. The patient will undergo gait analysis.
3. The patient will have an MRI of the knee

Evaluation at 6 months:

The patient will be contacted to fill out WOMAC and WOMET surveys.

<u>Evaluation at 12 months: (Primary outcome time point)</u>

1. The patient will fill out WOMAC and WOMET surveys
2. The patient will undergo gait analysis

Beyond this time point, patients will be followed for an additional year to address the exploratory aims of the study, particularly those having to do with cartilage thinning and radiographic progression of osteoarthritis. We acknowledge that the information gathered from this point forward may be underpowered, but it will serve as preliminary data for future grant applications.

Evaluation at 18 months:

The patient will be contacted to fill out WOMAC and WOMET surveys.

Evaluation at 24 months:

1. The patient will fill out WOMAC and WOMET surveys.
2. The patient will have standing plain radiographs to assess for progression of osteoarthirtis
3. The patient will have MRI of the knee and thigh

Subjects will remain blinded with regards to treatment until the conclusion of the study. At 24 months will ask them if they learned of their treatment and if so, how.

The subjects will be compensated $100 for study-related travel each time they visit the laboratory.

Detailed Description of Study Techniques:

MRI timing – patient experience and control of activity prior to scan:

Subjects will not have performed any strenuous activity for two hours prior to the MRI. Subjects will always undergo MRI testing following gait testing. Thus, all subjects will have similar mechanical conditions prior to their MRI. This will reduce the potential for confounding short-term effects that could influence articular cartilage thickness measurements.

MRI Sequences to be used in this study

Table 2: MR Imaging Protocol at 3.0 T

Sequence	Scan Time	OA Marker
2D IWFSE	4 min	Bone Marrow Lesions, synovitis, effusion, menisci, ligaments
3D T 1 ρ	8 min	Cartilage GAG
3D-DESS	8 min	Cartilage Thickness, 3D Cartilage T2 Diffusion Map
3D Sodium	25 min	Cartilage GAG

The imaging protocol at 3.0T (Table 2) in these subjects will last one hour, and including qDESS imaging with T_2, diffusion, and cartilage and joint morphology, $T_{1\rho}$ mapping as described above, and sodium MRI. To assess morphology as well as bone marrow edema, we will use intermediate-weighted (IW) 2D-FSE with fat suppression {Boileau, 2008}. Long-term assessment of regional cartilage thickness, as well as additional assessment of cartilage, menisci, and ligaments will be performed using DESS imaging {Eckstein, 2006;Guermazi, 2009}, the current method used in the Osteoarthritis Initiative. This baseline morphologic

imaging capability gives us the same capacity to follow joint changes using post-processing for cartilage volume, regional cartilage thickness changes, and internal derangement scored by BLOKS {Hunter, 2008;Peterfy, 2004;Felson, 2010}.

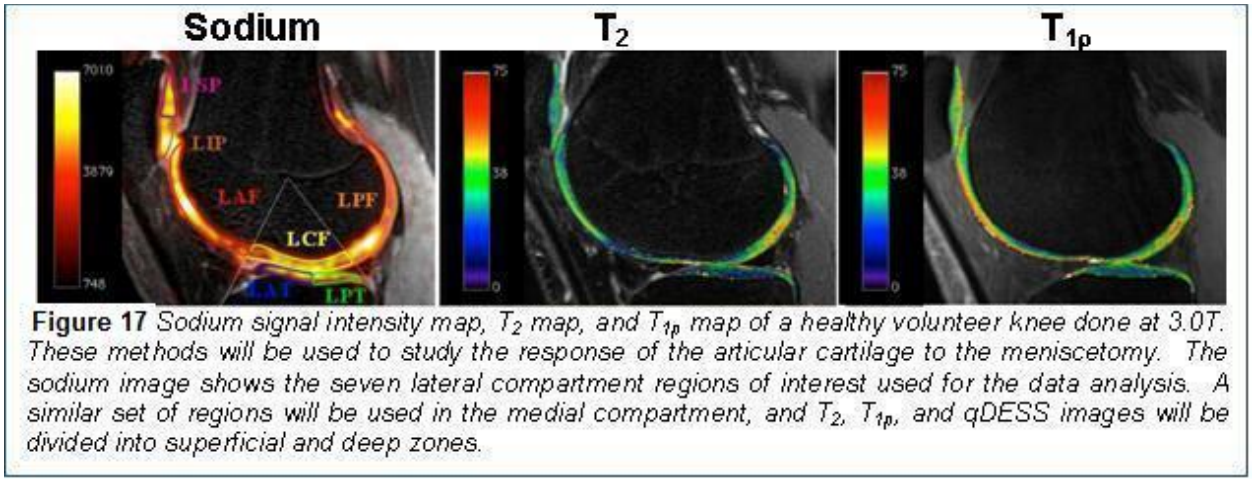


Figure 17 *Sodium signal intensity map, T_2 map, and $T_{1\rho}$ map of a healthy volunteer knee done at 3.0T. These methods will be used to study the response of the articular cartilage to the meniscetomy. The sodium image shows the seven lateral compartment regions of interest used for the data analysis. A similar set of regions will be used in the medial compartment, and T_2, $T_{1\rho}$, and qDESS images will be divided into superficial and deep zones.*

We will compare and correlate the results of multiple standard imaging markers of OA with our new methods. All (established and novel) sequences in this protocol will have varying degrees of change in the post-menisctomy population (Figure 17). We will directly compare *in vivo* sodium, diffusion, and $T_{1\rho}$ imaging for sensitivity to GAG changes in cartilage in a moderate number of OA patients. We will analyze the T_2 maps obtained from qDESS to assess regional variations of the collagen matrix. Diffusion weighted MRI is anticipated to show greater sensitivity to changes in the GAG matrix, so this may be a leading indicator of matrix change. The data from these comparisons will be rich, allowing us to correlate changes in cartilage GAG with bone marrow edema, meniscal pathology, and changes seen in in T_2 and ADC using qDESS.

MR Image Segmentation :
The first step of creating surface models from MR images is delineating boundaries of objects in two-dimensional slices. The boundary detection of an object in image has been one of important subjects in computer vision field. Our approach to this open and extensively studied problem is a semi-automatic method, similar to techniques described in the literature [Falcão, 1998, Stammberger, 1999, Steines, 2000]. We use a B-Spline Snake method with manual initialization to detect the cartilage boundary on a single MR image. In order to fit the B-spline snake to the image data, an energy minimization algorithm is employed. The cost function is composed of internal energy, image energy and constraint energy. Accuracy and precision of this cartilage thickness measurement technique has been studied and reported (Koo et al., 2009). Ability to see changes in cartilage thickness at the two-year time point has been demonstrated previously (Wirth et al., 2009).

Gait Analysis
Gait analysis in this study will be performed with a point cluster technique (Dyrby and Andriacchi, 2004; Alexander and Andriacchi 2001). The point cluster technique was developed to better account for skin movement during gait analysis. Skin movement relative to the underlying bone is a primary factor limiting the resolution of detailed joint movement using skin-based system. In most cases only large motions such as flexion extension have acceptable error limits with skin-based marker systems. Skeletal

movement can be measured using alternative approaches to a skin-based marker system. These approaches include electrogoniometers, bone pins external fixation devices and single plane fluoroscopic techniques. All of these methods are invasive or expose the test subject to radiation. Therefore, the widespread applicability of these methods is limited. A method described as a point cluster technique (PCT) permits direct *in vivo* measurement of the six-degree of freedom motion of the femur on the tibia while performing activities of daily living. A key feature of PCT method relative to fluoroscope or other imaging methods is the ability to acquire unencumbered motion measurements. Artifacts associated with the laboratory environment causing the subject to walk in an unnatural manner are a major potential source of error in the interpretation of human movement. The method proposed in this study avoids placing fixtures tightly strapped to the limb segments or requiring the subject to move in a limited fixed field of view required by fluoroscopic, radiographic or magnetic resonance imaging techniques.

Gait Analysis Protocol: Each subject will be tested standing in an anatomical position, during level walking and finally during stair ascent and descent. The level walking measurements include six stride cycles for each side over a range of walking speeds. The subject is instructed to walk at a comfortable speed (normal), slower than normal and faster than normal. Typically, this protocol produces gait measurements over a range of walking speeds. The stair climbing protocol consists of walking up a set of steps with a rise of 21cm and run of 23cm. The first step of the staircase is placed on the forceplate in order to record the forces during the lifting motion of stairclimbing. Again, three trials for each leg will be captured for ascent and descent.

The instrumentation to capture the skin-based marker data includes an eight camera opto-electronic system for 3-D motion analysis, a multi-component force plate for measurement of foot-ground reaction force and a computer system for acquisition, processing and analysis. Multiple video cameras acquire data with the subject during activities of interest. The over-abundance of markers (Figure 18) on each limb segment is used to define a cluster coordinate system (Figure 19), which is tied to an anatomically relevant coordinate system calculated with the subject at rest.

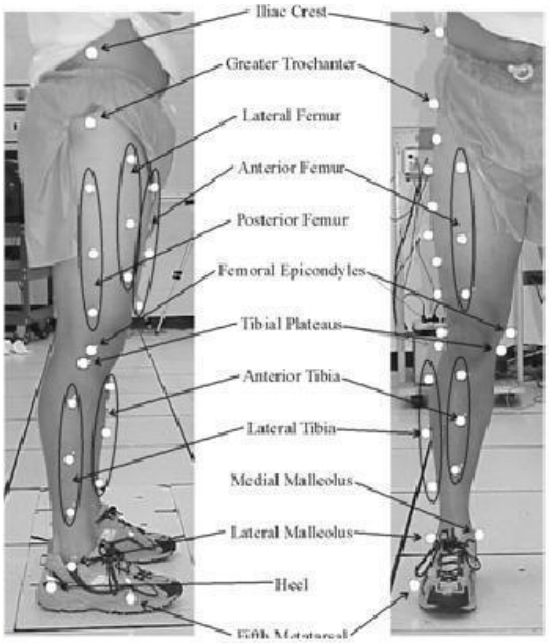


Figure 18 Markers placement for the Point Cluster Technique

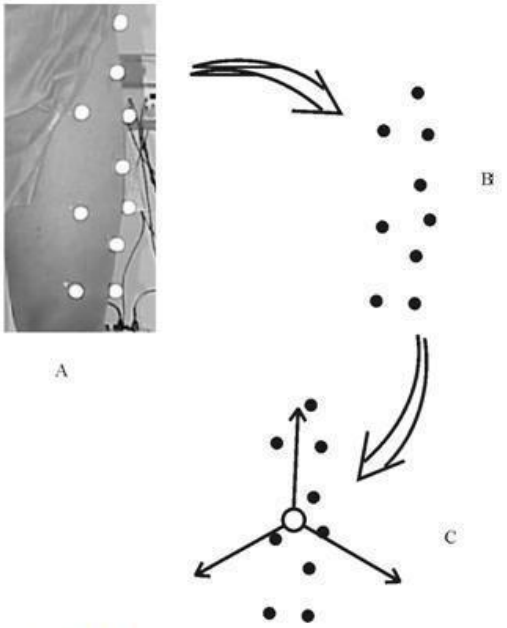


Figure 19 Resolving the cluster coordinate system. A) Shows the cluster on the thigh segment. This is resolved into three dimensional points (B) which then create the cluster coordinate system (CCS) C.

Nine markers are placed directly over bony landmarks and fifteen others evenly distributed on the anterior and lateral portions of the thigh and shank (Figure 18). Techniques described in the literature are used to locate these bony landmarks. The femoral medial-lateral axis runs through the trans-epicondylar line (a line drawn between the medial-lateral femoral epicondyles). The midpoint of this axis is the origin. The inferior-superior axis runs parallel to the long axis of the femur, passing through the midpoint of the trans-epicondylar line. The anterior-posterior axis is the cross product of the medial-lateral and inferior-superior axes. The final position of the inferior-superior axis is made orthogonal to the anterior-posterior and medial-lateral axis. For the shank segment, the tibial coordinate system begins with the medial-lateral axis running through the most medial and lateral edges of the plateau. The inferior-superior axis is perpendicular to the medial-lateral axis passing through the tibial eminence. The anterior-posterior axis is the cross product of the medial-lateral and inferior-superior axes.

Kinetic and kinematic data is acquired through an eight camera motion analysis system with Qualisys MCU 240 cameras controlled by Qualisys Track manager software. A Computer Boards DAS-1602 analog to digital converter card provides synchronized force data from a Bertek 4060-10 force plate. The software and hardware are housed on a Pentium based personal computer, with 2 GB RAM and 60 GB hard disk space. The eight cameras are positioned in an arc approximately 1.5 meters from the center of the measurement volume on one side of a 10 meter walkway.

Gait analysis will be carried out at three months after meniscectomy, and again at 12 months after meniscectomy. The three month gait analysis data on peak adduction moment and positioning of the tibia with respect to the femur will give us an idea of how quickly changes are manifest, but will not be the primary outcome time point.

Sensitivity of MRI to detect articular cartilage thickness changes

We have investigated whether MRI can detect articular cartilage changes in a 1-4 year time window following soft tissue injury to the knee. In this particular study, ACL injury was the model, and the purpose of this study was to test the feasibility that cartilage thinning (relative to the healthy contralateral knee) could be detected in period of approximately 4 years or less past ACL injury in reconstructed knees.

Twelve subjects with unilateral ACL reconstructions and no other history of serious lower limb injury (avg 38 yrs, 1.7 m, 68 kg, 4 male, 3.0 mo injury to reconstruction, 9-48 mo past reconstruction) were recruited for the study. All subjects were reconstructed within 6 months after injury. Subjects underwent bilateral, non-weightbearing 1.5T MR imaging. Tibial articular cartilage were segmented from knee MR images and reconstructed into three-dimensional (3D) cartilage thickness maps and the cartilage models from the right knee were mirrored and registered to the cartilage models from the left knee using surface matching techniques. The thickness maps were compared point-by-point and made into difference maps. The thinning volume in the weight-bearing region of the medial tibial plateau, normalized by its surface area, was calculated for the ACL reconstructed knees.

The results (Figure 20) suggest that articular cartilage thinning can be detected relatively early (15 months) after soft tissue injury to the knee. These preliminary data show that our MR-based measurement of cartilage thinning has adequate sensitivity to detect early patterns of morphological changes after ACL injury. These methods will be applied in this study towards investigating articular cartilage thickness changes after meniscal injury and meniscectomy.

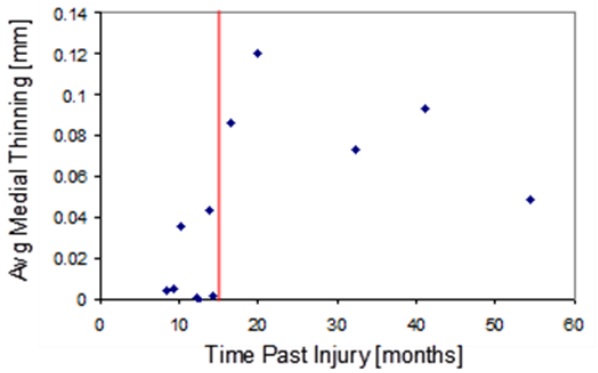


Figure 20. Medial tibial cartilage thinning with time after ACL injury in ACL reconstructed patients. The vertical red line is at 15 months after injury.

Biomarker analysis of synovial and serum samples

We will test the hypothesis that increased complement activation, levels of inflammatory cytokines and/or levels of cartilage metabolism biomarkers are associated with accelerated cartilage loss. Biomarkers of cartilage metabolism (Table 3 below) as well as inflammatory cytokines (Table 4 below) will be measured as indicated. The rationale for characterizing inflammatory cytokines, including IL-1□, IL-6 and TNF, is based on the putative role of these cytokines in driving low-grade inflammation that might contribute to the pathogenesis of OA. Studies on human samples and in mouse models have suggested a role for IL-1□ and other cytokines in OA pathogenesis. Further, IL-1□ and other cytokines modulate neural signals arising from painful stimuli and may thereby exacerbate gait dysfunction in OA. The rationale for analyzing complement components is based on data from our laboratory (Wang et al, 2011) demonstrating elevations of complement components in OA synovial fluid and tissue, and on our data demonstrating that C5-deficient mice are protected against OA (Wang et al, 2011). The cartilage degradation product COMP have been shown by Dr. Andriacchi's

laboratory to be elevated following exercise, and certain markers of cartilage degradation or synthesis are thought to reflect cartilage turnover, disease severity, or disease progression in OA. The metalloproteinases (MMP) 3 and 13 mediate the proteolytic digestion and subsequent breakdown of cartilage in OA. For certain markers, such as certain collagen degradation products, there are findings suggesting that urine provides a more reliable sample source as compared to serum. We will use the sample source previously demonstrated to be most robust for detection of the biomarker. We will use commercially available ELISA/EIAs (Tables below) to measure the levels of select complement components, cytokines, and cartilage metabolism biomarkers in serum and synovial fluid samples.

Table 3. Markers of cartilage metabolism to be measured in serum and/or synovial fluid (SF).

Description	Biological fluid to be tested	Symbol	Units	Average (Standard deviation)	Kit source
Cartilage oligomeric matrix protein	serum	**COMP**	U/L	12.62(4.2)	Pacific Biomarkers
Hyaluronic acid	serum	**HA**	ng/ml	11.4(4.5)	Corgenix
Chondroitin sulfate 846	serum	**CS-846**	ng/ml		Ibex
Matrix metalloproteinase-3	serum, SF	**MMP-3**	ng/ml	64(199)	RnD Systems
Matrix metalloproteinase-13	serum, SF	**MMP-13**	ng/ml	54(18)	RnD
Type II collagen neoepitope	Serum	**C2C**	ng/ml		Systems
Keratan sulfate	serum	**KS-5D4**	ng/ml	27(19)	Ibex

Table 4. Markers of complement activation and inflammation.

Description	Biological fluid to betested	Symbol	Units	Average (Standard deviation)	Kit source
High-sensitivity C-reactive protein	serum	**hsCRP**	mg/L	2.5(1.7)	Hemagen Diagnostics
Tumor necrosis factor-alpha	serum, SF	**TNF**	pg/ml	12.97(23.52)	Bio-Rad
Interleukin-1β	serum, SF	**IL-1β**	pg/ml	2.74 (2.77)	Bio-Rad
Interleukin-6	serum, SF	**IL-6**	pg/ml	0.09 (0.8)	Bio-Rad
Complement C3a	Serum, SF	**C3a**	ng/ml	103.3 (19.7)	BD, OptEIA
Complement C5b-9	SF	**MAC**	ng/ml		BD, OptEIA

Multiplex cytokine analysis. The rationale for multiplex cytokine profiling stems from the putative role of cytokines in driving low-grade inflammation, which might contribute to the pathogenesis of OA. Studies on human samples and in mouse models have suggested a role for IL-1□ and other cytokines in OA pathogenesis. Further, IL-1□ and other cytokines modulate neural signals arising from painful stimuli and may thereby exacerbate gait dysfunction in OA. We identified a panel of cytokines that are elevated in severe OA (KL=4) compared to less severe OA (KL<4) (Fig. 3.5). Using protocols optimized by the Robinson Laboratory (Hueber et al, 2007), we will use a 50-cytokine BioPlex bead array (Table 5 below) and the Luminex System (the Robinson Lab possesses a Luminex200 system and a Bio-Rad automated magnetic bead washing system) to measure the levels of 50 cytokines in all synovial fluid and serum samples collected.

Table 5. The 50-cytokine panel to be measured by BioPlex bead array.									
IL-1β	IL-6	IL-12	IL-16	MIP-1b	IL-1α	Eotaxin	CTACK	LIF	β-NGF
IL-1ra	IL-7	IL-13	IL-18	PDGF-bb	IL-2Ra	FGF basic	GROa	MCP-3	SCF
IL-2	IL-8	IL-15	IP-10	RANTES	IL-3	G-CSF	HGF	M-CSF	SCGF-β
IL-4	IL-9	IL-17	MCP-1	TNF-α	TRAIL	GM-CSF	ICAM-1	MIF	SDF-1α
IL-5	IL-10	IL-12p40	MIP-1α	VEGF	VCAM-1	IFN-γ	IFN-α2	MIG	TNF-β

Power calculations & bioinformatic analysis. We will test the hypothesis that increased complement activation, cartilage metabolism and/or inflammatory biomarkers are associated with accelerated cartilage loss. We will explore use of (i) predefined indexes of markers of cartilage metabolism (Table 3 above) and markers of inflammation (Table 4 above), and (ii) a combined multivariate test. For the predefined indexes of markers, we will standardize each marker to have standard deviation 1, so an average of 8 independent markers would have a variance 1/8, and an average of 6 a variance of 1/6. For the combined multivariate testing for the cartilage metabolism and the inflammatory markers, we will use 8 and 6 degrees of freedom, respectively, in conjunction with a multivariate Hotelling's T-test. Power calculation: So to achieve an effect (difference in mean index score) of .25 standardized units using a two-sample-test at (0.05 two-sided, power 0.8) would require 29 samples in each group for the 8-marker index, and 43 per group for the 6-marker index.

Statistical Analysis

Randomization:

We will explicitly test if randomization achieved balance between the experimental groups on potentially confounding variables such as age, gender, and body weight. However, confounding may still operate even if the differences between the groups are not statistically significant. Therefore, we will conduct two secondary analyses in which imbalances in important potential confounding variables (duration of symptoms, grade of chondromalacia found on arthroscopic evaluation of the joint, radiographic severity of arthritis (Kellgren-Lawrence), and diagnosis of depression) will be addressed. First, we will simply add these covariates to the primary regression model. However, estimating so many more parameters will inherently reduce the statistical power of the test of the intervention effect. Our second approach solves this problem by first calculating a propensity score (the probability of being in the meniscectomy group based on pre-treatment variables) for each patient, and then using the propensity scores as regression weights in the main regression analysis. This is a state of the art approach for estimating treatment effects in the presence of potential confounding.

Aim 1: *To test the clinical benefit of arthroscopic meniscectomy in the setting of a stable degenerative meniscus tear.*

Hypothesis 1a: The 12 month WOMAC score will be our primary outcome measure for this aim and the endpoint for this power analysis. The WOMAC is a validated osteoarthritis outcome tool that is widely used to assess the severity of pain, stiffness, and function due to osteoarthritis. A 20% improvement in the total WOMAC score is considered to be clinically important (Kirkley et al., 2008). In a preliminary study, we evaluated a cohort of 45 patients undergoing arthroscopy and meniscectomy at our institution (Mirza et al., 2010). We found a mean preoperative WOMAC score of 1296 with a standard deviation of 392. Twenty percent of the mean baseline score of 1296 is 260.

To ensure patient safety, we are incorporating in our analysis an interim look at the results by the DSMB to ensure that one group does not have dramatically superior results to another group. In addition, the DSMB will assess subject attrition rates by group and adverse events.

This will take place after 25% of the total planned number of subjects complete the study. This analysis will include assessment of drop-out rates and adverse events as well.

Setting the experiment-wide alpha at 0.05 and power at 80%, sample sizes of 37 and 37 achieve 81% power to detect a difference of 260 between the group means with standard deviations of 392 and 392 at a significance level (alpha) of 0.05 using a two-sided independent sample t-test. These results assume that 2 sequential tests are made using the O'Brien-Fleming spending function to determine the test boundaries. The interim look will thus take place when the first 20 subjects (25% of the total 74) complete the 12 month assessment. The analysis assumes that we will stop the trial if either condition is superior. The critical t-value will at the interim look will be +/- 4.3326. If the trial continues, the final analysis will use a critical t-value of 1.96. Sensitivity analyses will be conducted with t-tests on the baseline-to-12-month change scores.

74 subjects will thus be needed to complete the trial. Over a one year period we will expect to have a 10% lost to follow-up rate. We will thus plan to enroll 82 subjects into the study. With a 70% recruitment rate we will need to have 117 candidate patients for this study. In calendar year 2011, we performed 66 arthroscopic meniscectomies at our facility. Assuming that 70% of these cases involved stable degenerative tears, we would have 46 candidate patients per year. It would thus take us approximately 2.5 years to recruit enough patients for the study if we were to operate at last year's rate. We presently have approximately 100 patients for knee arthroscopy on our waiting list for surgery, which makes it likely that we could accomplish this goal more rapidly.

Hypothesis 1b: Should we fail to find a difference in 12 month WOMAC scores, we will test the hypothesis that the WOMAC scores in the arthroscopy and lavage condition are at least as good as (non-inferiority) the menisecomy by setting "d" the largest difference in WOMAC scores which we consider to be clinically meaningless to 15% of baseline, or 194 points. We will then calculate 90% confidence limits for the difference in means for the two groups and determine whether these confidence limits lie within the -194 to 194point interval. Since there is no danger to patients if this hypothesis is found to be true, this hypothesis will not be subject to an interim analysis. We will assume that the standard deviation of the means is 392. With these numbers, the sample size of 74 and alpha = .10 and yields power = .80.

Aim 2: *To test the functional effect of arthroscopic meniscectomy in the setting of a degenerative meniscus tear.*

Hypothesis 2: Subjects randomized to arthroscopy and meniscectomy will have greater changes in tibio-femoral rotation, knee adduction moment, and knee flexion moment at 12 months following surgery when compared to subjects randomized to arthroscopy and lavage.

Calculations were performed to estimate the necessary sample size for the endpoints of Aim 2 of changes in tibio-femoral rotation, knee adduction moment, and knee flexion moment. In order to keep the family-wise error rate at .05, each comparison will be made with an independent sample t-test with alpha = 0.0167. To reject the null hypothesis that changes in tibio-femoral rotation will not differ between the intervention (meniscectomized) knees and control (non-meniscectomized knees), data from a similar study performed in our laboratory evaluating tibial rotation using the same techniques between meniscectomized knees and intact knees was used (Netravali et al., 2010). In that study, we found a 3.2 degree difference in tibio-femoral rotation in meniscectomized knees when compared to intact contralateral knees using a t-test. The standard deviation of the measurements in the meniscectomized knees was 3.8 degrees. Thus, to detect a difference of 3.2 degrees tibio-femoral rotation in the meniscectomized group

versus no change in the intact group, with an alpha of 0.0167 and 74 total patients, power of 87% will be achieved.

In addition, we would be interested to detect a change in peak adduction moment of about 10% of baseline value. This corresponds to a change of 0.35%BW*ht (Netravali et al., 2010). Prior work looking at changes in knee adduction moment with intervention find a standard deviation of the difference in peak adduction moment with the intervention to be 0.35%BW*ht. With an alpha of 0.0167 and 74 total patients, power of 94% will be achieved.

Finally, we are interested in detecting a change in peak flexion moment of about 10% baseline value. This corresponds to a change of 0.3%BW*ht (Netravali et al., 2010). Assuming a standard deviation of the flexion moment of approximately 0.35%BW*ht. With an alpha of 0.0167 and 74 total patients, power of 89% will be achieved.

Missing Data:
All analyses will be on the intent-to-treat sample. To address missing data at 1 year (and other time points), we will use a multiple imputation strategy that minimizes potential attrition bias. Ten datasets will be imputed with draws from the theoretical complete data distribution using the Multivariate Imputation by Chained Equations (MICE) program implemented in the R statistical language. All analyses with these data will be conducted on the imputed datasets and combined to yield the final parameter estimates and inferences.

Timeline and Targeted/Planned Enrollment Table:

Timeline (months)	**Time Frame**	**2**	**5**	**6**	**10**	**12**	**14**	**18**	**22**	**24**	**26**	**32**	**35**	**36**	**44**	**46**	**48**
Organize and train personnel	0-2 mo	xxxxx															
Secure IRB and R&D Comm Approvals	0-2 mo	xxxxx															
Screen and recruit subjects	2-32 mo		xxxxx	xxxx	xxxx	xxxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx					
Three month follow up evalutations	5-35 mo			xxxx	xxxx	xxxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx				
One year follow up evaluations (primary)	14-44 mo							xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx		
Perform interim analysis	Approx 22 mo								X								
Data analysis of primary outcomes	44-46 mo															xxx	
Information dissemination	46-48 mo																xxxx
Two year follow up evaluations (exploratory)	26-end											xxxx	xxxx	xxxx	xxxx	xxx	xxxx
Follow-on grant preparation	46-48 mo																xxxx
Subject screening goal	117 total	0	12	16	31	39	47	62	78	86	94	117					
Subject recruitment goal	82 total	0	8	11	22	27	33	44	55	60	66	82					
Subject primary aim completion goal	**74 total**						**0**	**10**	**20**	**25**	**29**	**44**	**52**	**54**	**74**		
Exploratory aim completion											0	15	22	25	44	49	54

Note: we will assess the success of our recruitment at the end of 10 months and double our rate of surgery (and thus our opportunities for recruitment at the preoperative evaluation) if we find ourselves behind in recruitment efforts.