Effect of intraoperative application of autologous PRP on post operative morbidity in ACL reconstruction using autologous bone patellar tendon bone graft harvest

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Introduction

Platelet-rich plasma (PRP) has been advocated as a way to introduce increased concentrations of growth factors and other bioactive molecules to injured tissues in an attempt to optimize the local healing environment. PRP has been used extensively in dental and cosmetic surgery for almost 30 years and its safety and efficacy in these areas is well established. Recently, there has been increasing evidence that the increased levels of autologous bioactive proteins provided by the concentrated platelets in PRP could enhance tissue repair and regeneration in a variety of orthopaedic pathologies (4). Currently PRP is being used in almost all areas and pathologies in orthopaedics with a paucity of high-level evidence to support its use. Initially popularized for its effectiveness in the treatment of lateral epicondylitis and other tendinopathies (1,9,5), PRP's indications have recently broadened to acute conditions such as muscle or ligamentous injury and ankle sprains (2,11) where it has been proven effective at decreasing pain. ACL repair using autologous BPTB grafts is widely used in ACL reconstruction due its excellent healing potential and lack of risk of disease transmission that accompanies cadaveric graft use. The main drawback or poor outcome in autologous BPTB reconstruction has been donor site morbidity manifest as anterior knee pain and pain with kneeling activity. Animal studies and clinical results of the use of PRP in ACL reconstruction have been mixed but do show some promise in the areas of graft incorporation and donor site morbidity (7,8,10,12,13). There is currently a paucity of well-designed, prospective, randomized, blinded (Level 1) clinical studies that carefully and comprehensively investigate the role of PRP in ACL surgery. Specifically, ACL reconstruction using BPTB harvest and the role of PRP in decreasing postoperative pain at the donor site. Given the need for a highly powered study to demonstrate the clinical efficacy of PRP in ACL reconstruction, we have designed a prospective, randomized, level 1, double-blinded study to evaluate the effects of PRP on the specific outcomes of donor site pain and radiographic measures of graft healing and incorporation.

Specific Aim

The aim of this study is to evaluate the efficacy of intra-operatively applied autologous PRP in reducing donor site morbidity and increasing radiographic healing in ACL reconstruction using autologous BPTB.

Drug Information

Platelet-rich plasma (PRP) is blood plasma that has been enriched with platelets. As a concentrated source of autologous platelets, PRP contains (and releases through degranulation) several different growth factors and other cytokines that stimulate healing of bone and soft tissue. The efficacy of certain growth factors in healing various injuries and the concentrations of these growth factors found within PRP are the theoretical basis for the use of PRP in tissue repair. The platelets collected in

PRP are activated by the addition of thrombin and calcium chloride, which induces the release of these factors from alpha granules. The growth factors and other cytokines present in PRP include:

- Platelet-derived growth factor
- Transforming growth factor beta
- Fibroblast growth factor 1
- Insulin-like growth factors 1,2
- Vascular endothelial growth factor
- Epidermal growth factor
- Interleukin 8
- Keratinocyte growth factor
- Connective tissue growth factor

There are, at present, two methods of PRP preparation approved by the U.S. Food and Drug Administration. Both processes involve the collection of whole blood that is anti-coagulated with citrate dextrose before undergoing two stages of centrifugation designed to separate the PRP aliquot from platelet-poor plasma and red blood cells. In humans, the typical baseline blood platelet count is approximately 200,000 per uL. Therapeutic PRP concentrates the platelets by roughly five-fold. There is however broad variability in the production of PRP by various concentrating equipment and techniques.

In humans, PRP has been investigated and used as clinical tool for several types of medical treatments, including nerve injury, tendinitis, cardiac muscle injury, bone repair and regeneration, plastic surgery, and oral surgery. PRP has also received attention in the popular media as a result of its use in treating sports injuries in professional athletes. Currently, no PRP type reagents are being used in patients undergoing autologous BPTB ACL reconstruction as a part of the standard care of patients.

Taking all of the above into account and in keeping with current handling in orthopaedic surgeries where PRP is handled, the Arthrex ACP System for harvesting and preparing PRP under sterile conditions in the OR will be used. This will require the donation of approximately 10cc of patients venous blood drawn prior to the start of the case-in the OR, after patients have been placed under general anesthesia, and under sterile conditions. The Arthrex ACP system will be utilized to prepare the sample and the 3-5ml harvest of supernatant will be utilized to apply the PRP at the end of the case.

The equipment needed: ACP/Double Syringe with Cap, Anticoagulant ACD-A: 50ml, Centrifuge, rotor set, Bucket, Bucket Cap, and counterbalance will be provided by Arthrex.

Research and Design Methods:

A. Study Design: This is a single-center, prospective, randomized, double-blinded study utilizing patients undergoing ACL reconstruction with autologous bone patellar tendon bone graft harvest under the care of one of two surgeons (SJN or BB) at Surgicare of Manhattan.

B. Inclusion Criteria:
   - Primary ACL Reconstruction
   - Outerbridge </= 2 at time of surgery
   - Minimum follow up of two years
   - No ligamentous secondary injury
   - Willingness to participate in the study, including follow up at regular intervals and completion of subjective questionnaires

C. Exclusion Criteria:
   - Any previous knee injury
   - Any previous history of patellofemoral pain/anterior knee pain
   - Outerbridge classification 3 or higher
   - Revision ACL
   - Diabetic or smoker
   - Workers compensation patient
   - Any patient with limited English proficiency
   - Pregnant or nursing women

D. Patient Enrollment: Enrollment will be based on the above inclusion criteria. We plan to enroll approximately 100 patients with 50 randomized to the treatment arm, and 50 to the control. Our power analysis was based upon previous studies on post-operative morbidity in patients undergoing ACL reconstruction with autologous bone patellar tendon bone graft harvest. To establish significance, we need 35 patients per group to detect a 20% difference in anterior knee pain with an α = .05 and a power of 80%. Assuming a loss to follow up of up to 30% at two years, adequate sample recruitment was determined to be 50 per group or 100 total. (This calculation and appropriate references are described in this protocol: section I; pg 21)

E. Recruitment Procedure: Patients undergoing an ACL reconstruction with autologous BPTB graft harvest will be asked prior to surgery for their participation in this study after considering the inclusion/exclusion criteria. Informed consent will be obtained any time after the patient is booked for surgery (usually 2-3 weeks before surgery) and before the day of surgery (not on the day of surgery). Patients will be blinded to treatment randomization.

F. Data Collection: Data collection will be prospective

- Pre-operative data collection requiring patients to fill out a form will include: the IKDC Demographic Form (see below) which will require approximately 5 minutes of time during a preoperative office visit.

IKDC Demographic Form

IKDC DEMOGRAPHIC FORM

Your Full Name _____

Your Date of Birth _____/_____/_____
Day Month Year

Your Social Security Number ____-___-_____ **Your Gender:** ❑ **Male** ❑ **Female**

Occupation _____

Today's Date _____/_____/_____
Day Month Year

The following is a list of common health problems. Please indicate "Yes" or "No" in the first column, and then skip to the next item. If you do have the problem, please indicate in the second column if you receive medications or some other type of treatment for the problem. In the last column, indicate if the problem limits any of your activities.

	Do you have the problem?		Do you receive treatment for it?		Does it limit your activities?	
	Yes	No	Yes	No	Yes	No
Heart disease	❑	❑	❑	❑	❑	❑
High blood pressure	❑	❑	❑	❑	❑	❑
Asthma or pulmonary disease	❑	❑	❑	❑	❑	❑
Diabetes	❑	❑	❑	❑	❑	❑
Ulcer or stomach disease	❑	❑	❑	❑	❑	❑
Bowel disease	❑	❑	❑	❑	❑	❑
Kidney disease	❑	❑	❑	❑	❑	❑
Liver disease	❑	❑	❑	❑	❑	❑
Anemia or other blood disease	❑	❑	❑	❑	❑	❑
Overweight	❑	❑	❑	❑	❑	❑
Cancer	❑	❑	❑	❑	❑	❑
Depression	❑	❑	❑	❑	❑	❑
Osteoarthritis, degenerative arthritis	❑	❑	❑	❑	❑	❑
Rheumatoid arthritis	❑	❑	❑	❑	❑	❑
Back pain	❑	❑	❑	❑	❑	❑
Lyme disease	❑	❑	❑	❑	❑	❑
Other medical problem	❑	❑	❑	❑	❑	❑
Alcoholism	❑	❑	❑	❑	❑	❑

IKDC Demographic Form p.2

Page 2 - IKDC DEMOGRAPHIC FORM

1. Do you smoke cigarettes?

   ❑Yes
   ❑No, I quit in the last six months.
   ❑No, I quit more than six months ago.
   ❑No, I have never smoked.

2. Your height ❑centimeters ❑inches

3. Your weight ❑kilograms ❑pounds

4. Your race (indicate all that apply)

   ❑White ❑Black or African-American ❑Hispanic

   ❑Asian or Pacific Islander ❑Native American Indian ❑Other

5. How much school have you completed?

   ❑Less than high school ❑Graduated from high school ❑Some college

   ❑Graduated from college ❑Postgraduate school or degree

6. Activity level

   ❑Are you a high competitive sports person?

   ❑Are you well-trained and frequently sporting?

   ❑Sporting sometimes

   ❑Non-sporting

- Pre–operative objective data collection will include:
    - Time from injury to surgery, and a radiographic grading of osteoarthritis using the Kellgren-Lawrence classification scale performed by one senior musculoskeletal radiologist (DK).
- Intra-operative data collection will include: Outerbridge classification of medial, lateral, patellofemoral compartments osteoarthritis, blood loss, time of surgery, tourniquet time, meniscal pathology and its treatment. A sample of intraoperative data sheets is listed below:

2000 IKDC SURGICAL DOCUMENTATION FORM

Patient's Name: _____ Date of Index Procedure: _____/_____/_____
Day Month Year

Postoperative Diagnosis:

1._____

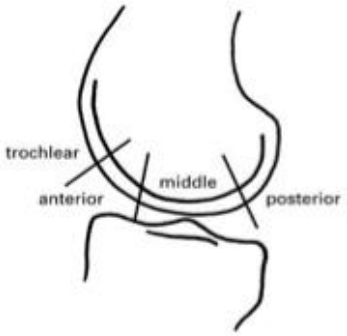
2._____

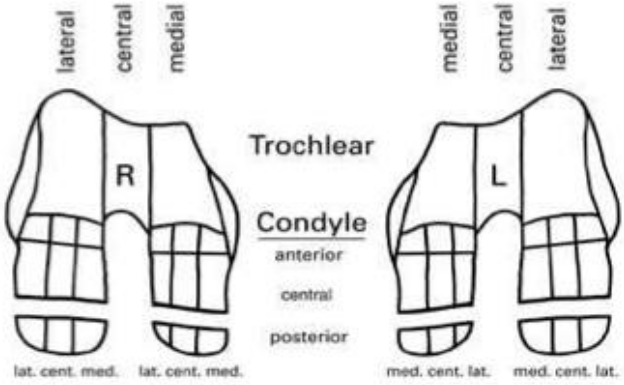
3._____

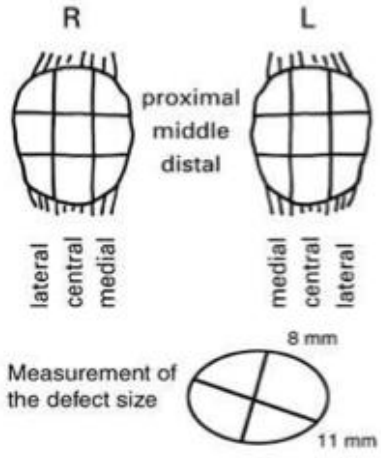
Status After Procedure:

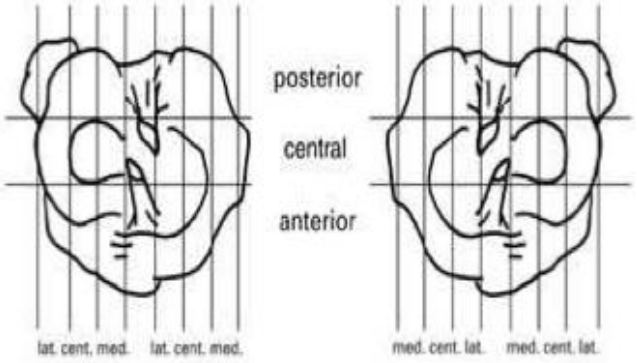
ARTICULAR CARTILAGE STATUS:

Document the size and location of articular cartilage defects on these figures according to the ICRS mapping system[c].









Page 2 – 2000 IKDC SURGICAL DOCUMENTATION FORM

Record size, location and grade of articular cartilage lesions.

Femur

					First lesion	Second Lesion
Side	Right	Left				
Condyle	Medial	Lateral				
Sagittal plane	Trochlear	Anterior	Middle	Posterior		
Frontal plane	Lateral	Central	Medial			
Cartilage lesion (Grade) (*)						
Defect size pre-debridement			mm			
Defect size post-debridement			mm			

Tibia

					First lesion	Second Lesion
Side	Right	Left				
Plateau	Medial	Lateral				
Sagittal Plane	Anterior	Middle	Posterior			
Frontal Plane	Lateral	Central	Medial			
Cartilage lesion (Grade) (*)						
Defect size pre-debridement			mm			
Defect size post-debridement			mm			

Patella

					First lesion	Second Lesion
Side	Right	Left				
Sagittal plane	Distal	Middle	Proximal			
Frontal plane	Lateral	Central	Medial			
Cartilage lesion (Grade) (*)						
Defect size pre-debridement			mm			
Defect size post-debridement			mm			

Diagnosis: ❑ Traumatic cartilage lesion ❑OD ❑OA ❑AVN ❑Others

Biopsy/Osteochondral Plugs: Location: Number of Plugs:

Diameter of Plugs: mm

Treatment:
❑ Shaving ❑ Abrasion
❑ Drilling ❑ Microfracture
❑ Osteochondral autograft transfer/mosaic-plasty
❑ Cell therapy ❑ Other

Notes:

ICRS Grade 0 - Normal

ICRS Grade 1 – Nearly Normal
Superficial lesions, Soft indentation (A) and/or superficial fissures and cracks (B)

A B

ICRS Grade 2 – Abnormal
Lesions extending down to <50% of cartilage depth

ICRS Grade 3 - Severely Abnormal
Cartilage defects extending down >50% of cartilage depth (A) as well as down to calcified layer (B) and down to Blisters are included in this Grade (D)

A B

C D

ICRS Grade 4 – Severely Abnormal
Osteochondral injuries, lesions extending just through the subchondral boneplate (A) or deeper defects down into trabecular bone (B). Defects that have been drilled are regarded as osteochondral defects and classified as ICRS-C.

A B

Page 3 – 2000 IKDC SURGICAL DOCUMENTATION FORM

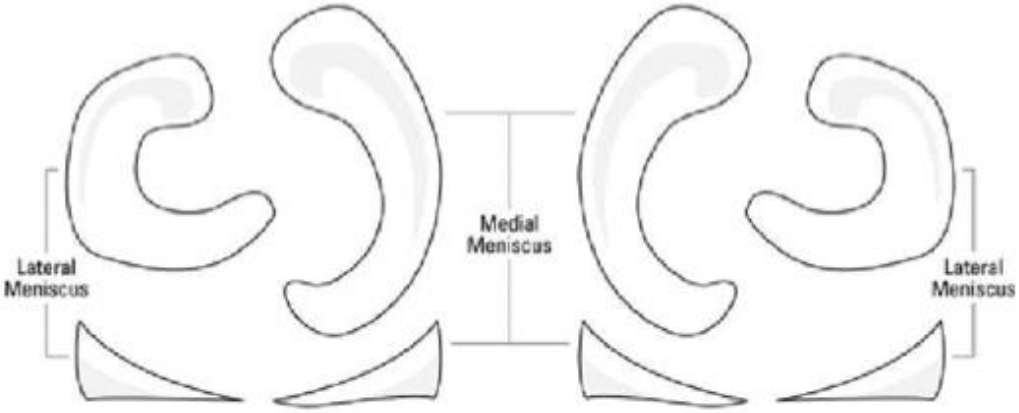
MENISCUS STATUS:

Procedure:

❑medial meniscectomy	❑lateral meniscectomy
❑medial meniscal repair	❑lateral meniscus repair
❑medial meniscal transplant	❑lateral meniscal transplant
❑medial abrade & trephine	❑lateral abrade & trephine

Right Knee **Left Knee**

Document tears of the menisci or meniscectomy on these figures



Medial:

❑Normal ❑1/3 Removed ❑2/3 Removed ❑3/3 Removed

Circumferential Hoop Fibers: ❑Intact ❑Disrupted

Remaining Meniscal Tissue: ❑Normal ❑Degenerative changes
❑Stable tear ❑Unstable tear
❑Tear left in situ

Lateral:

❑Normal ❑1/3 Removed ❑2/3 Removed ❑3/3 Removed

Circumferential Hoop Fibers: ❑Intact ❑Disrupted

Remaining Meniscal Tissue: ❑Normal ❑Degenerative changes
❑Stable tear ❑Unstable tear
❑Tear left in situ

Page 4 – 2000 IKDC SURGICAL DOCUMENTATION FORM

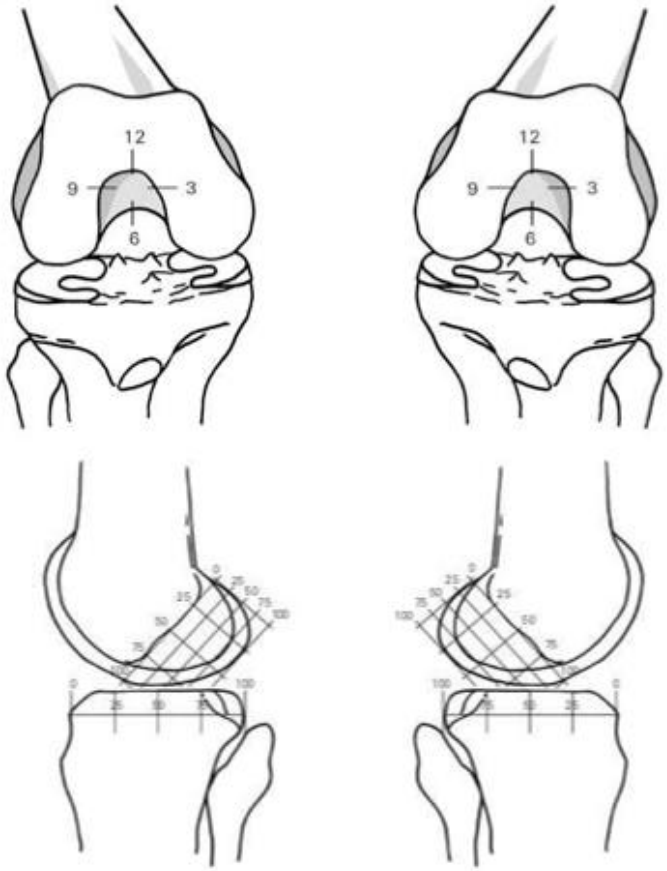
LIGAMENT STATUS:

Procedure:
❑ACL repair ❑Intraarticular ACL reconstruction ❑Extraarticular ACL reconstruction
❑PCL repair ❑Intraarticular PCL reconstruction ❑Posterolateral corner repair/reconstruction
❑Medial collateral ligament repair/reconstruction
❑Lateral collateral ligament repair/reconstruction

Graft:
❑Autologous patella tendon ❑Hamstring tendons ❑Quadriceps tendon
❑Other_____

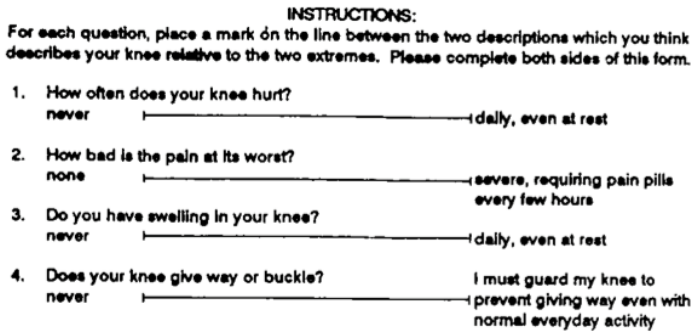
Previous Graft Harvest:
❑Autologous patella tendon ❑Hamstring tendons ❑Quadriceps tendon

Document drill hole placement for ligament reconstruction on these figures.



- Post-operative data collection will include both subjective and objective outcomes measures.
    - Subjective outcomes of donor site morbidity will include VAS for ADL's, VAS for kneeling, IKDC subjective knee evaluation form, and a return to sports/subjective performance questionnaire. Each post operative office visit will require approximately 10 minutes of patient time to fill out the subjective questionnaires.
        - Collected at 14days and 1,3,6,12,18 and 24 months

Example of how a VAS questionnaire is converted to numerical data points:



INSTRUCTIONS:
For each question, place a mark on the line between the two descriptions which you think describes your knee relative to the two extremes. Please complete both sides of this form.

1. How often does your knee hurt?
   never — daily, even at rest
2. How bad is the pain at its worst?
   none — severe, requiring pain pills every few hours
3. Do you have swelling in your knee?
   never — daily, even at rest
4. Does your knee give way or buckle?
   never — I must guard my knee to prevent giving way even with normal everyday activity

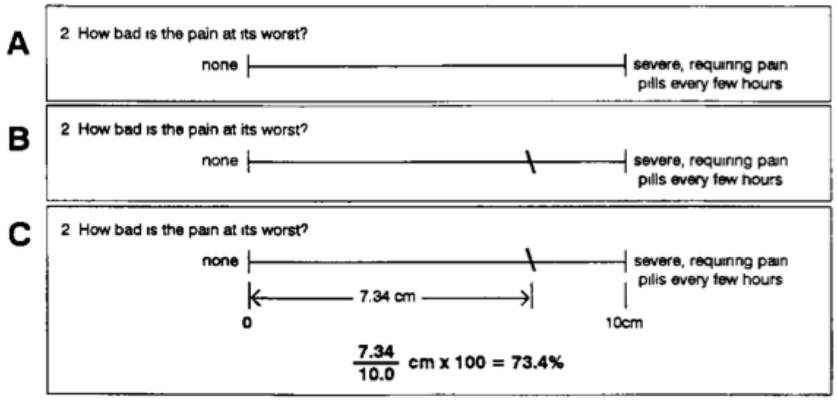
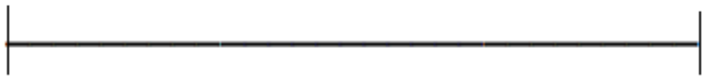


Figure 1. A, a VAS for recording the severity of pain. The question posed would read, "How bad is the pain at its worst?" B, the patient places a mark at what he feels is the appropriate point on the line. C, through simple measurement techniques, this graphic representation may be converted to a numerical value.

Visual Analog Scale
Activities of Daily Living

For each question, place a mark on the line between the two descriptions which you think describes your knee relative to the two extremes.

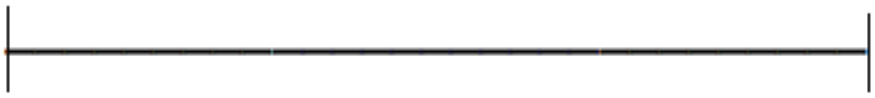


No Knee Discomfort
With Activities of Daily

Constant Knee Pain
With Activities of Daily

Visual Analog Scale
Kneeling Pain

For each question, place a mark on the line between the two descriptions which you think describes your knee relative to the two extremes.



No Knee Discomfort With Kneeling

Unable to Kneel Due to Knee Pain

IKDC Subjective Knee Evaluation Form

2000 IKDC SUBJECTIVE KNEE EVALUATION FORM

Your Full Name_____

Today's Date: _____/_____/_____ (Day / Month / Year) Date of Injury: _____/_____/_____ (Day / Month / Year)

SYMPTOMS*:

*Grade symptoms at the highest activity level at which you think you could function without significant symptoms, even if you are not actually performing activities at this level.

1. What is the highest level of activity that you can perform without significant knee pain?

   4 ❑ Very strenuous activities like jumping or pivoting as in basketball or soccer
   3 ❑ Strenuous activities like heavy physical work, skiing or tennis
   2 ❑ Moderate activities like moderate physical work, running or jogging
   1 ❑ Light activities like walking, housework or yard work
   0 ❑ Unable to perform any of the above activities due to knee pain

2. During the past 4 weeks, or since your injury, how often have you had pain?

	10	9	8	7	6	5	4	3	2	1	0	
Never	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	Constant

3. If you have pain, how severe is it?

	10	9	8	7	6	5	4	3	2	1	0	
No pain	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	Worst pain imaginable

4. During the past 4 weeks, or since your injury, how stiff or swollen was your knee?

   4 ❑ Not at all
   3 ❑ Mildly
   2 ❑ Moderately
   1 ❑ Very
   0 ❑ Extremely

5. What is the highest level of activity you can perform without significant swelling in your knee?

   4 ❑ Very strenuous activities like jumping or pivoting as in basketball or soccer
   3 ❑ Strenuous activities like heavy physical work, skiing or tennis
   2 ❑ Moderate activities like moderate physical work, running or jogging
   1 ❑ Light activities like walking, housework, or yard work
   0 ❑ Unable to perform any of the above activities due to knee swelling

6. During the past 4 weeks, or since your injury, did your knee lock or catch?

   0 ❑ Yes 1 ❑ No

7. What is the highest level of activity you can perform without significant giving way in your knee?

   4 ❑ Very strenuous activities like jumping or pivoting as in basketball or soccer
   3 ❑ Strenuous activities like heavy physical work, skiing or tennis
   2 ❑ Moderate activities like moderate physical work, running or jogging
   1 ❑ Light activities like walking, housework or yard work
   0 ❑ Unable to perform any of the above activities due to giving way of the knee

Page 2 – 2000 IKDC SUBJECTIVE KNEE EVALUATION FORM

SPORTS ACTIVITIES:

8. What is the highest level of activity you can participate in on a regular basis?

4❑Very strenuous activities like jumping or pivoting as in basketball or soccer
3❑Strenuous activities like heavy physical work, skiing or tennis
2❑Moderate activities like moderate physical work, running or jogging
1❑Light activities like walking, housework or yard work
0❑Unable to perform any of the above activities due to knee

9. How does your knee affect your ability to:

		Not difficult at all	Minimally difficult	Moderately Difficult	Extremely difficult	Unable to do
a.	Go up stairs	4❑	3❑	2❑	1❑	0❑
b.	Go down stairs	4❑	3❑	2❑	1❑	0❑
c.	Kneel on the front of your knee	4❑	3❑	2❑	1❑	0❑
d.	Squat	4❑	3❑	2❑	1❑	0❑
e.	Sit with your knee bent	4❑	3❑	2❑	1❑	0❑
f.	Rise from a chair	4❑	3❑	2❑	1❑	0❑
g.	Run straight ahead	4❑	3❑	2❑	1❑	0❑
h.	Jump and land on your involved leg	4❑	3❑	2❑	1❑	0❑
i.	Stop and start quickly	4❑	3❑	2❑	1❑	0❑

FUNCTION:

10. How would you rate the function of your knee on a scale of 0 to 10 with 10 being normal, excellent function and 0 being the inability to perform any of your usual daily activities which may include sports?

FUNCTION PRIOR TO YOUR KNEE INJURY:

Couldn't perform daily activities	0 ❑	1 ❑	2 ❑	3 ❑	4 ❑	5 ❑	6 ❑	7 ❑	8 ❑	9 ❑	10 ❑	No limitation in daily activities

CURRENT FUNCTION OF YOUR KNEE:

Cannot perform daily activities	0 ❑	1 ❑	2 ❑	3 ❑	4 ❑	5 ❑	6 ❑	7 ❑	8 ❑	9 ❑	10 ❑	No limitation in daily activities

- Objective outcomes measures will be collected in the form of radiographic and clinical data collection. (Appendix C)

    - Triple view radiographic series of the knee at 3 months postop to assess tunnel placement and donor site healing by one blinded senior radiologist (DK).

    - Post-op MRI at 6 months to assess degree of donor site healing. Interpreted by one blinded senior musculoskeletal radiologist (DK)

    - Objective clinical assessments will include IKDC knee examination form scores, ROM measured with goniometer, and quadriceps strength. This data will be collected by an independent researcher (RR, SS) also blinded to treatment. In order to protect the blinding of the study, the senior surgeon(s) (SJN or BB) will examine the patients post operatively but their assessments will not be included in the clinical data collection.

Data Collection Sheet- Objective

Pt. ID #	XR tunnel placement	XR Donor Site Healing	MRI Donor Site Healing	IKDC	Quadriceps Strength	ROM
001						
002						
003						
004						
005						

IKDC Objective Knee Evaluation Form

2000
IKDC KNEE EXAMINATION FORM

Patient Name:_____ **Date of Birth:**_____/_____/_____ (Day / Month / Year)

Gender: F M **Age:**_____ **Date of Examination:**_____/_____/_____ (Day / Month / Year)

Generalized Laxity:	tight	normal	lax	
Alignment:	obvious varus	normal	obvious valgus	
Patella Position:	obvious baja	normal	obvious alta	
Patella Subluxation/Dislocation:	centered	subluxable	subluxed	dislocated
Range of Motion (Ext/Flex):	Index Side: passive_____/_____/_____		active_____/_____/_____	
	Opposite Side: passive_____/_____/_____		active_____/_____/_____	

SEVEN GROUPS	**FOUR GRADES** A Normal	B Nearly Normal	C Abnormal	D Severely Abnormal	*Group Grade A B C D
1. Effusion	None	Mild	Moderate	Severe	
2. Passive Motion Deficit					
ΔLack of extension	<3°	3 to 5°	6 to 10°	>10°	
ΔLack of flexion	0 to 5°	6 to 15°	16 to 25°	>25°	
3. Ligament Examination (manual, instrumented, x-ray)					
ΔLachman (25° flex) (134N)	-1 to 2mm	3 to 5mm(1+) <-1 to –3	6 to 10mm(2+) <-3 stiff	>10mm(3+)	
ΔLachman (25° flex) manual max Anterior endpoint:	-1 to 2mm firm	3 to 5mm	6 to 10mm soft	>10mm	
ΔTotal AP Translation (25° flex)	0 to 2mm	3 to 5mm	6 to 10mm	>10mm	
ΔTotal AP Translation (70° flex)	0 to 2mm	3 to 5mm	6 to 10mm	>10mm	
ΔPosterior Drawer Test (70° flex)	0 to 2mm	3 to 5mm	6 to 10mm	>10mm	
ΔMed Joint Opening (20° flex/valgus rot)	0 to 2mm	3 to 5mm	6 to 10mm	>10mm	
ΔLat Joint Opening (20° flex/varus rot)	0 to 2mm	3 to 5mm	6 to 10mm	>10mm	
ΔExternal Rotation Test (30° flex prone)	<5°	6 to 10°	11 to 19°	>20°	
ΔExternal Rotation Test (90° flex prone)	<5°	6 to 10°	11 to 19°	>20°	
ΔPivot Shift	equal	+glide	++(clunk)	+++(gross)	
ΔReverse Pivot Shift	equal	glide	gross	marked	
4. Compartment Findings			crepitation with		
ΔCrepitus Ant. Compartment	none	moderate	mild pain	>mild pain	
ΔCrepitus Med. Compartment	none	moderate	mild pain	>mild pain	
ΔCrepitus Lat. Compartment	none	moderate	mild pain	>mild pain	
5. Harvest Site Pathology	none	mild	moderate	severe	
6. X-ray Findings					
Med. Joint Space	none	mild	moderate	severe	
Lat. Joint Space	none	mild	moderate	severe	
Patellofemoral	none	mild	moderate	severe	
Ant. Joint Space (sagittal)	none	mild	moderate	severe	
Post. Joint Space (sagittal)	none	mild	moderate	severe	
7. Functional Test					
One Leg Hop (% of opposite side)	≥90%	89 to 76%	75 to 50%	<50%	
****Final Evaluation**					

G. Experimental Design

Patients will be randomized using a computer generated randomization table (simple randomization) into one of two treatment arms:

(1) Autologous BPTB ACL reconstruction followed by application of PRP soaked bone chips = Treatment

(2) Autologous BPTB ACL reconstruction = Control

The patient, research personnel and individuals collecting all objective outcome measures will be blinded to the randomization. The operating surgeons (SJN or BB) will evaluate the subjects pre and post operatively but will not contribute any of their objective data collection to the study. Randomization and patient assignments will be done through sequentially numbered opaque sealed envelopes by the circulating nurse in the OR at the time of surgery. The randomization will not be stratified according to surgeon but rather-according to treatment arm. Randomization information will then be recorded and placed into a sealed envelope with the patients' randomization ID and deposited into a locked reception box. This box will only be opened upon completion of the study.

H. Research Procedure

ACL reconstruction

Will be performed using standard approved surgical technique. The surgeries will be performed by one of two senior surgeons (SJN or BB) and diagnostic arthroscopy will verify the inclusion criteria are met (no additional ligamentous injury, no osteochondral defect, and Outerbridge classification grade 2 or lower). After fixation of the graft and just prior to closure, the senior attending (SJN or BB) and any other research personnel present will leave the room so that application of either PRP soaked cancellous bone chips or non-treated cancellous bone chips to the graft harvest site can be performed in a manner that will maintain blinding. A surgical fellow or resident who will not be present for any portion of the outcomes data collection will do this and therefore, will not compromise the blinding of the study. The Paratenon will be closed over the material thus sealing it within the donor site and the wound will be closed using normal guidelines.

PRP Preparation

The PRP will be prepared directly in the operating room prior to the surgical intervention but after the patient has been placed under general anesthesia. The Arthrex ACP Double Syringe system will be used according to their manufacturing guidelines. The blood sample will be obtained by the anesthesiologist at the start of the case, just after the patients have been placed under general anesthesia via the same IV access utilized to administer the anesthesia. The Anesthesiologist will have no role in the data collection or surgical intervention aspect of the study. As the blood will be drawn after patients are asleep, they will remain blinded to their treatment arm and only those in the treatment arm will have blood withdrawn and no additional IV access will be necessary.

For each patient in the treatment arm of the study, the anesthesiologist will withdraw 10cc of venous blood prior to the surgeon or research assistants having entered the room. The blood sample will be given to an OR assistant to prepare prior to the start of the case. The 10cc of venous blood will be mixed together with 1 cc of ACD-A (citrate anticoagulant) and centrifuged for 5 min at 1500 rpm. The blood sample will separate into red blood cells and a supernatant "buffy coat" layer containing platelets (PRP) and white blood cells. This Leukocyte rich prp layer will be approximately 3-5cc in volume and will be aspirated into the inner smaller diameter syringe of the double syringe system. Lastly, the PRP phase will be mixed together with .25cc of $CaCl_2$ and placed within a sterile specimen cup that is covered in aluminum foil so that no one can see what is inside. Once the graft has been harvested, the autologous cancellous bone chips retrieved will be added to the specimen cup by the scrub technician. The PRP and cancellous bone chips will be mixed together in a separate sterile preparatory area of the operating room. They will be left to soak within the concealed container until the end of the case when the chips and PRP will be placed together within the patellar bony defect. A separate member of the staff who is not involved in any of the postoperative data collection measures will do this. Prior to placement of the PRP soaked cancellous chips within the wound and closure, the primary surgeon (SJN or BB) and any personnel present who will also be present during the post operative time frame for outcomes measurements will leave the room.

Concealing the contents of the specimen cup, preparing the PRP and adding it to the specimen cups prior to the presence of any active participant in the study (Senior surgeon or otherwise) entering the room, and adding the prp soaked bone chips to the patellar defect without any active study members in the room ensures blinding.

I. Statistical Analysis/Data Management

We plan to enroll approximately 100 patients with 50-treatment/50 controls. Power analysis was based upon previous studies examining kneeling pain using a VAS in patients undergoing ACL reconstruction with Autologous Bone Patellar Tendon Bone graft harvest. Feller et al (2001) reported kneeling pain to be 4.5+/-2.1 4 mo. after surgery, Wipfler et al (2011) reported kneeling pain to be 1.6+/-0.7 1 yr after surgery, and Siebold et al (2006) reported kneeling pain to be 4.0+/-3.1 3.8 yr after surgery (mean +/-SD reported). A 2-point difference in kneeling pain between groups would be regarded as clinically significant. The between subject SD for kneeling pain of averaged 2.0 for the 3 studies cited above with an upper limit of approximately 3.0. Therefore we conservatively based the sample size estimate to demonstrate a 2.0 difference in VAS pain between groups on a SD of 3.0. It is estimated that with 35 patients per group there will be 80% power to detect a 20% difference in kneeling pain (2 points on a 10 point scale) at an alpha level of 0.05. This estimate was calculated using the equation described by Kirkwood et al (1988).

Assuming a loss to follow up of up to 30% at two years, adequate sample recruitment was determined to be 50 per group or 100 total.

References Cited for Power Analysis

1: Siebold R, Webster KE, Feller JA, Sutherland AG, Elliott J. Anterior cruciate ligament reconstruction in females: a comparison of hamstring tendon and patellar tendon autografts.
Knee Surg Sports Traumatol Arthrosc. 2006 Nov;14(11):1070-6.
Epub 2006 Jun 7. PubMed PMID: 16758236.

2: Wipfler B, Donner S, Zechmann CM, Springer J, Siebold R, Paessler HH. Anterior cruciate ligament reconstruction using patellar tendon versus hamstring tendon: a prospective comparative study with 9-year follow-up.
Arthroscopy. 2011 May;27(5):653-65. PubMed PMID: 21663722.

3: Feller JA, Webster KE, Gavin B. Early post-operative morbidity following anterior cruciate ligament reconstruction: patellar tendon versus hamstring graft.
Knee Surg Sports Traumatol Arthrosc. 2001 Sep;9(5):260-6. PubMed PMID:11685356.

4:Kirkwood BR (1988). Calculation of required sample size. In Essentials of Medical Statistics. pp 191-200. Oxford. Blackwell Scientific Publications.

The secondary outcome measures are objective and include radiographic evidence of graft site healing and clinical objective measurements. The primary and secondary outcomes measures for the two groups will be statistically analyzed to evaluate for any differences between the two groups over time using mixed model analysis of variance with Group (PRP vs. no PRP) and the between subjects factor and Time (14d, 1,3,6,12,18 and 24 months post op) as the within subjects factor. Bonferroni corrections will be used for between group or between time pair-wise planned comparisons.

The de-identified data will be entered into an Excel spreadsheet and maintained on a password-protected computer. Once data collection is complete, the principal investigator (SJN) and/or his research assistant (MM) will destroy the master hard copy of identifier codes. Confidentiality of data, data integrity, review of data and study quality will be monitored by the principal investigator and/or his research assistant. Baseline data, demographic data, primary and secondary outcomes will be analyzed using summary statistics, standard deviations and 95% confidence intervals for quantitative data. Qualitative data will be assessed using frequencies and percentages.

J. Informed Consent:
Informed valid written consent will be obtained from all patients at the time of enrollment. This will be in accordance to IRB consent forms

K. Potential Risks & Discomforts:

a. *Platelet Rich Plasma*: Since PRP is a formulation created entirely from the participants own blood, the complications and risks are extremely low. The anesthesiologist who prepares the blood is highly skilled and will carefully sterilize the skin prior to obtaining the sample. Another side effect that is common is an initial increase in pain and inflammation at the site where the PRP is injected. This occurs because the platelets and blood sample contain natural chemicals that stimulate the immune system. This type of complication is short lived and when it does occur, is treated effectively with pain medication and anti-inflammatory medications, such as Ibuprofen. PRP is not recommended for individuals who have a known bleeding disorder (bleed easily) or are on a medication known to thin the blood such as Coumadin or Lovenox. This makes sense because these patients are more likely to bleed than an individual who has normal bleeding characteristics.
b. *Blood Draw*: There are no major risks of having blood drawn in this study. Participants will be fully anesthetized while the blood is drawn, which therefore mitigates the pain of the injection. In addition, the blood will be

drawn through the IV that is already being placed as part of their scheduled surgery. Therefore, participants will not experience any additional bruising or discomfort as a result of participating in this study.

c. *X-ray:* As a result of x-ray imaging, participants will be exposed to radiation. Since having x-rays is a standard part of post ACL reconstruction surgery, participants will not be exposed to any additional radiation for participating in this study.
d. *Magnetic Resonance Imaging*: Since MRI machines use magnets and radio waves to take images of the body, participants are not subjected to any radiation. However, participants might experience discomfort with having to lie on a narrow enclosed couch for 15 minutes as the machine takes the images. In addition, the machine produces loud tapping noise that might be cause discomfort to the participants. The only caution for this test is to ensure that participants have no metal on or in their body since the machine might disrupt them.
e. *Survey:* There are no risks associated with completing the survey. Participants might feel discomfort from completing the survey as a result of the questions being asked, but they are reminded that they can stop completing them at any time.
f. *Breach of confidentiality*: Although it is unlikely, there is a small risk that there can be a breach of confidentiality if patient information is disclosed to unauthorized individuals. All measures to ensure patient confidentiality will be employed. Data will be de-identified and entered into a password-protected computer. Only the principal investigator (SJN) and research assistant (MM) will have access to their data with patient identifiers.

L. Data Safety Monitoring Board: The data safety monitoring plan for this study will involve patient follow-ups and interim analysis. At each follow-up session, the investigator will assess healing of both the donating and recipient sites. If any infections are present, they will be addressed as per standard of care which involves cleaning the area arthroscopically. The investigator will also perform an interim analysis after half of the accrual goal is achieved. At this point, study data will be reviewed by the investigator to ensure patient safety and the continued validity of the study.

M. Potential Benefits:
The participants randomized to receive PRP in this study may directly benefit by having decreased healing time and decreased post operative pain, specifically anterior knee pain common to patients in the postoperative time frame following autologous BPTB reconstruction of their ACL. The study may provide knowledge on the benefits of PRP in graft site healing and patient satisfaction following ACL reconstruction.

N. Discontinuation of Study/Subject Withdrawal

A patient may be withdrawn from the study due to lack of follow up or voluntary withdrawal. The study may also be discontinued at any time depending on the circumstances and any adverse events on the discretion of the investigating personnel and the IRB.

O. Adverse Events

If the patient has any adverse effects from being in the study, they will receive medical care and treatment as needed from the North Shore-Long Island Jewish Health System. However, the patient will be responsible for the costs of such medical treatment, directly or through their medical insurance and/or other forms of medical coverage. No money will be given to the patient. All adverse events will be reported as necessary, per NSLIJ IRB policy.

P. Confidentiality

Each subject will be assigned a unique number in consecutive order, not derived from any patient identifiers, known as a code number. These code numbers will be listed on a "Master Subject Log" and will serve to link the subject's code number with their name, (date of birth and medical record number). The code numbers will be the only patient identifier collected on the Data Collection Sheet. At the conclusion of this investigation, the Master Subject Log will be destroyed to ensure patient confidentiality. Data will be compiled in a Microsoft Excel spreadsheet which will be password protected to maintain patient confidentiality. All other study documents will be kept in the office of Stephen J. Nicholas. There will be no disclosure of the PHI. Paper copies of the study documents will be created only as needed and shown only to the investigators listed in the study personnel and will be destroyed immediately after they are no longer needed. Patient data (unidentified) will be contained on a password-protected computer. Only the investigators will have access to the data. In the event that the results of this study are published or used in any presentations, all information will be de-identified. It will be impossible to recognize individuals.

Q. References

1. de Vos RJ, Weir A, van Schie HT, Bierma-Zeinstra SM, Verhaar JA, Weinans H, Tol JL. Platelet-rich plasma injection for chronic Achilles tendinopathy: a randomized controlled trial. JAMA. 2010 Jan 13;303(2):144-9.

2. Everts et al Exogenous application of platelet-leukocyte gel during open subacromial decompression contributes to improved patient outcome. A prospective randomized double-blind study. Eur Surg Res. 2008

3. Fleming BC, Spindler KP, Palmer MP, Magarian EM, Murray MM. Collagen-platelet composites improve the biomechanical properties of healing anterior cruciate ligament grafts in a porcine model. Am J Sports Med. 2009;37(8):1554-63. PMCID: 2796133.

4. Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB, Rodeo SA. Platelet-rich plasma: from basic science to clinical applications. Am J Sports Med. 2009;37(11):2259-72.

5. Gosens T, Peerbooms JC, van Laar W, den Oudsten BL. Ongoing positive effect of platelet-rich plasma versus corticosteroid injection in lateral epicondylitis: a double blind randomized controlled trial with 2-year follow-up. Am J Sports Med. 2011 Jun;39(6):1200-8.

6. Joshi SM, Mastrangelo AN, Magarian EM, Fleming BC, Murray MM. Collagen-platelet composite enhances biomechanical and histologic healing of the porcine anterior cruciate ligament. Am J Sports Med. 2009;37(12):2401-10. PMCID: 2856313.

7. Nin JR, Gasque GM, Azcarate AV, Beola JD, Gonzalez MH. Has platelet-rich plasma any role in anterior cruciate ligament allograft healing? Arthroscopy. 2009;25(11):1206-13.

8. Orrego M, Larrain C, Rosales J, Valenzuela L, Matas J, Durruty J, Sudy H, Mardones R. Effects of platelet concentrate and a bone plug on the healing of hamstring tendons in a bone tunnel. Arthroscopy. 2008;24(12):1373-80.

9. Peerbooms JC, Sluimer J, Bruijn DJ, Gosens T. Positive effect of an autologous platelet concentrate in lateral epicondylitis in a double-blind randomized controlled trial: Platelet-rich plasma versus corticosteroid

injection with a 1-year follow-up. Am J Sports Med. 2010 Feb;38(2):255-62.

10. Radice F, Yanez R, Gutierrez V, Rosales J, Pinedo M, Coda S. Comparison of magnetic resonance imaging findings in anterior cruciate ligament grafts with and without autologous platelet- derived growth factors. Arthroscopy. 2010;26(1):50-7.

11. Randelli PS, Arrigoni P, Cabitza P, Volpi P, Maffulli N. Autologous platelet rich plasma for arthroscopic rotator cuff repair. A pilot study. Disabil Rehabil. 2008;30(20-22):1584-9.

12. Sanchez M, Anitua E, Azofra J, Prado R, Muruzabal F, Andia I. Ligamentization of tendon grafts treated with an endogenous preparation rich in growth factors: gross morphology and histology. Arthroscopy. 2010;26(4):470-80.

13. Silva A, Sampaio R. Anatomic ACL reconstruction: does the platelet-rich plasma accelerate tendon healing? Knee Surg Sports Traumatol Arthrosc. 2009;17(6):676-82.

14. Spindler KP, Murray MM, Carey JL, Zurakowski D, Fleming BC. The use of platelets to affect functional healing of an anterior cruciate ligament (ACL) autograft in a caprine ACL reconstruction model. J Orthop Res. 2009;27(5):631-8. PMCID: 2752673.

APPENDIX A

Data Collection Sheet- Demographics

Pt ID #	SEX	AGE	BMI	OC	TTI
001					
002					
003					
004					
005					

Demographic characteristics

* Age
* Sex (female/male)
* Weight (kg)
* Height
* Outerbridge Classification (OC)
* Estimated time from injury to surgery (Time To Intervention TTI)

APPENDIX B

Data Collection Sheet- Subjective

Pt. ID #	VAS ADL	VAS Kneeling	RSQ	IKDC
001				
002				
003				
004				
005				

Return to Sports Questionnaire

- 6months post op

Visual Analog score for ADL

- 14d, 1, 3, 6, 12, 18, 24 months follow up

Visual Analog score for Kneeling:

- 14d, 1,3,6,12,18, 24 months follow up

IKDC subjective knee evaluation form

- 14d, 1,3,6,12,18, 24 months follow up

APPENDIX C

Data Collection Sheet- Objective

Pt. ID #	XR tunnel placement	XR Donor Site Healing	MRI Donor Site Healing	IKDC	Quadriceps Strength	ROM
001						
002						
003						
004						
005						

Radiographic healing

- 3 months – XR assessment of tunnel placement and donor site healing
- 6 months - MRI Assessment of Donor Site Healing

IKDC Knee examination form

- 14d, 1,3,6,12,18 and 24 months follow up

ROM Measurements

- 14d, 1,3,6,12,18 and 24 months follow up

Quadriceps Strength

- Dynamometry scale