Pilot Study: Augmentation of ACL Reconstruction with Bone Marrow Stem Cells and Amnion Collagen Matrix Wrap

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Study Design: Randomized Controlled Trial

No. 1 Background /Scientific Rationale

Histologic studies have determined that graft ligamentization following anterior cruciate ligament (ACL) reconstruction may take from 6 to 18 months. (1) It has been reported that incomplete graft maturation and incorporation is one cause of clinical graft failure. Animal studies have illustrated improved tendon healing/integration in ACL models augmented with stem cell technologies. (2-4). Basic scientists theorize that optimization of stem cell treatments for tissue regeneration requires that a "regenerative triad" be employed, i.e., use of a scaffold, stem cells and growth factors. In the intra-articular environment, research has shown that a scaffold such as an amnion wrap is necessary to contain the stem cells and growth factors in close proximity to the ACL graft. (2, 4, 5)

The normal, uninjured human ACL is covered by a layer of synovial tissue which contributes to the blood supply and nutrition of the native ACL. It is theorized that the lack of a synovial lining after injury and following traditional ACL reconstruction contributes to slow ligamentization and possible failure of reconstructed grafts.(5) Two studies have demonstrated accelerated maturation and ligamentization of human ACL graft augmented with point of care blood products. (6, 7) In one, leucocyte poor platelet rich plasma was injected directly into the body of the graft. (6) In the other, the platelet derived growth factors were loaded in a gelatin carrier which was wrapped around the graft. (7) In both studies accelerated and increased ligament maturation was documented compared to the controls. Collagen membranes derived from amniotic tissue have been successful to aid healing when used in difficult wounds and meniscal repair surgery. (8, 9)

We believe that use of a collagen based -membrane derived from amniotic tissue can be used to help reestablish the natural synovial lining of the reconstructed ACL, in effect acting as both a barrier from the synovial fluid and as a scaffold to contain autologous mesenchymal stem cells and growth factors contiguous with the graft, thus aiding and perhaps accelerating the natural maturation and ligamentization process of the implanted graft tissue. Acceleration and improvement in graft maturation and strength would be a significant advancement in sports medicine allowing safer and earlier return to sports and activity.

No. 2 Objectives

The objective of this study is to evaluate the use of wrapping an ACL graft with a collagen matrix tissue wrap and injecting autologous bone marrow aspirate concentrate under the wrapping and into the graft. We hypothesize that this method of augmenting ACL surgery will accelerate and improve the graft maturation and ligamentization process. We propose to test this hypothesis with a series of cases of ACL reconstructions evaluated with post-operative MRI mapping sequences and validated clinical outcome measures.

No. 3. Subject Eligibility

Inclusion Criteria: Patients between the ages of 18 and 35 who are scheduled to have anterior cruciate ligament reconstruction with autologous grafts by one of the investigating physicians will be screened for participation in this study. Patients must be willing to undergo MRI scans post –operatively at 3, 6, 9 months and 1 year.

Exclusion criteria: Patients with prior procedures or significant prior injuries to the same knee are excluded. Any patient who will have difficulty obtaining internet access, does not have an active e-mail address, or is unable to comprehend study documents or give informed consent will be excluded. Patient who are unable to complete MRI examinations due to claustrophobia or anxiety will be excluded.

No. 4. Subject Enrollment

Patients meeting the inclusion criteria will have the study explained to them by one of the members of the investigating team, and they will be given an opportunity to participate if they are interested. Up to 50 patients who agree to participate will go through the described informed consent process and be enrolled in the SOS registry, which is an IRB approved research database with Baptist Hospital that collects post-surgical outcome surveys. No specific advertising or recruitment material will be utilized.

No. 5. Study Design and Procedures

Study design will be a Level I prospective randomized and blinded trial. Subjects who meet the inclusion criteria will have the study explained in detail and informed consent will be obtained as outlined above. Subjects will be enrolled in the SOS knee arthroscopy registry and complete all pre-operative questions prior to the surgical date either on line or in the office with the assistance of the staff. The patient will complete all routine pre-operative, operative and post-operative protocols for the procedure. An Andrews Institute standard post-operative protocol for the procedure will be followed and physical therapy will be ordered for all participants per normal standard of care. Compliance in physical therapy will be documented.

The study will involve 20 patients undergoing ACL reconstruction with patellar tendon autografts and 20 patients with hamstring autografts for a total of 40 patients. During surgery the grafts will be harvested and prepared in the standard fashion. Patients undergoing ACL reconstruction with patellar and hamstring autografts will be randomized into 2 groups of 10 patients each. One group will have the collagen wrap and stem cell concentrate injection and one will have standard ACL surgery without augmentation of wrap and stem cell injection. All of these procedures are commonly performed at the Andrews Institute and standard protocols will be followed for all procedures. Patients will be blinded to the randomization results.

Bone marrow aspirate will be collected through the surgical site from the distal femur or the iliac crest with a trocar designed for this purpose. Therefore patients will be unable to determine if they have had stem cells harvested. The aspirate will be centrifuged using the Arthrex Angel system (see Appendix 1) to optimally concentrate the cells for implantation. The graft for the experimental groups will be wrapped with a sterile amnion matrix wrap, supplied by Arthrex (see attached Appendix 2), which will be sutured in place. (12) (Fig. 1) The grafts will be implanted and affixed using standard procedure. (Fig. 2) The mesenchymal stem cell concentrate will then be injected under direct visualization inside the collagen matrix wrap and into the graft construct. (Fig.3) A cell count of the stem cell concentrate will be performed prior to implantation to determine quality of the concentrate. After injection any remaining stem cell concentrate will be destroyed. Photographs will be taken to document the delivery of the cells into the graft construct. The grafts for the control groups will be implanted and affixed per normal procedure.

Both groups will be followed utilizing the SOS outcomes system protocol for 2 years. Both groups will undergo post-operative MRI at 3, 6, 9 months and one year utilizing a validated T2 star sequence which will undergo region of interest mapping to produce mean T2 values. (Fig. 4) These values have been shown to detect differences in ACL content, structure and maturation. Mapping will be performed by a blinded musculoskeletal radiologist. After the 12 month MRI, patients will be offered an office needle arthroscopy performed under local anesthesia for the purpose of gross and histological evaluation of their graft. An equal number from each randomized group will be recruited. The goal is to recruit 3 patients from each group, for a total of 12 patients.

No. 6 Expected Risks and Benefits

Risks and Discomforts: There have been no documented risks or side effects from using collagen membranes in human subjects. For patients randomized to the collagen wrap stem cell group, the stem cells will be harvested from either the femur or iliac crest during surgery. This will result in a small needle insertion hole in either the femur or iliac crest for the bone marrow stem cell collection, which will be about the same size as an IV insertion. This may cause some minor pain or swelling and there is a very minor risk of infection. The risks of needle arthroscopy include minor puncture site pain and infection, less than that published for standard arthroscopy. (13) There is a small risk of the exposure of patient's health information, which will be minimized through the rigorous requirements set up in the SOS IRB protocols and the use of subject codes in place of names on all data sheets.

Benefits: Patients may experience accelerated graft maturation as a result of agreeing to participate in the study. Some patients will receive at no extra charge mesenchymal stem cell augmentation of their ACL grafts. Patients will receive follow up MRI scans at no extra charge. Participation in the patient reported outcomes system gives the attending physician the opportunity to remotely monitor patient's health progress and outcomes. Future patients may benefit from the information obtained in this study.

No. 7. Data Management Procedures

All personal information is strictly confidential and no names will be disclosed except as required by law. All data sheets will have names and identifying information replaced with a subject code to protect the confidentiality of the patient. The principal investigator, co-investigators and research staff will have access to the de-identified data. All subject data forms will be scanned and stored, along with summary information forms and spreadsheets in a secure protected folder on a laptop to which only study investigators will have access. This data will be permanently deleted following any and all publications or presentations have been completed related to this research. All records related to this research will be retained in a secure location for a period of 3 years following the completion of all study related activities, after which time they will be properly destroyed.

No. 8. Data Analysis

The research committee will meet at appropriate intervals to evaluate and analyze the data. The investigators may also access the "Patient Analysis" panel on the SOS website at any time. This allows the investigator to perform comparative analysis on individual outcomes, institutional outcomes and the global de-identified average by choosing filters. De-identified data can be exported in raw data or graphical format.

No. 9. Statistical Considerations

This study will continue to enroll patients until 40 patients complete the study. The data will be arranged in Microsoft Excel spreadsheet format. Ranges, means and standard deviations for all measures will be determined and calculated. Common statistical procedures, such as ANOVA and analysis of covariance will be used to interpret changes in outcome variables from the survey results over time.

No. 10. Quality Control and Assurance

All protocols will be monitored and analyzed data will be checked for accuracy by the principal investigator and /or a designated AREF research team member. All medical data will be kept in compliance with HIPAA guidelines.

No. 11. Regulatory Requirements

Informed Consent: The informed consent process will be performed by one of the study investigators or staff, in the office at the time the decision for surgery is made. All subjects will have the study described to them and will give as much time as they require to read an approved, stamped version of the informed consent document. After signing of the informed

consent document, participants will be given a copy for their records. This process will take place only after the patient has consented to proceed with the surgical procedure.

Subject Confidentiality: Subject confidentiality information is listed above under #7. All medical data will be recorded and stored in compliance with HIPAA guidelines.

No. 12. References

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Figure 1: Graft preparation prior to implantation.

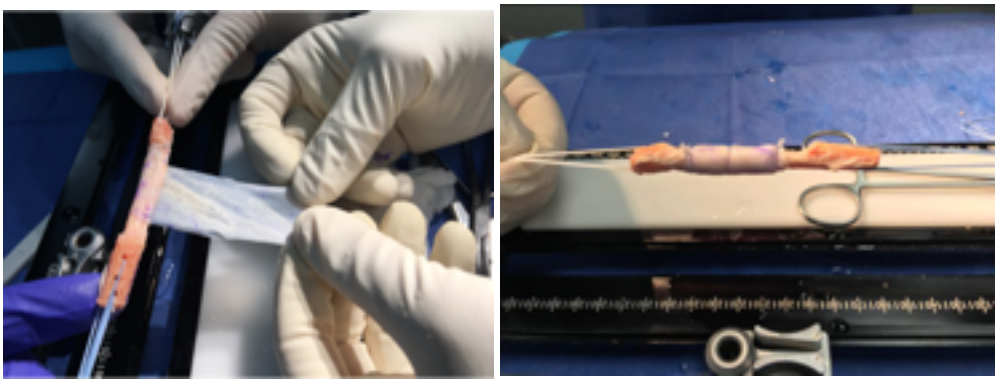

Figure 2: Graft implanted with surgeon's standard technique

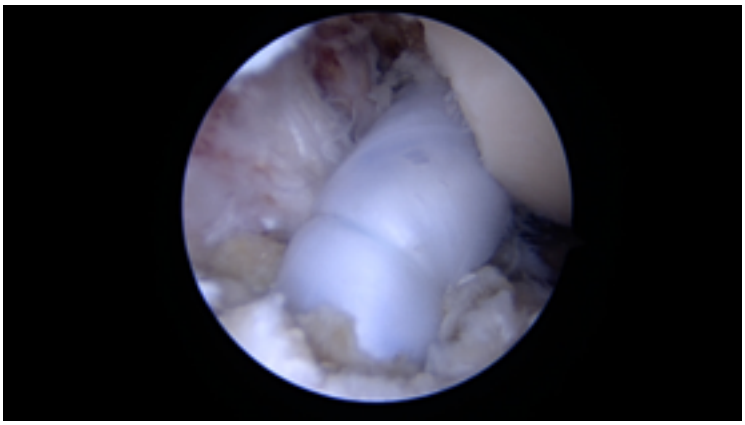

Figure 3: Injection of biologic under membrane

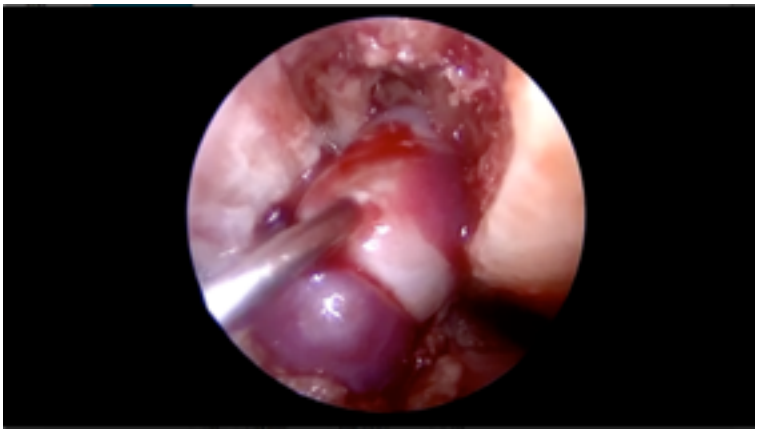

Figure 4: MRI mapping of ACL