Strategies to Improve Anterior Cruciate Ligament Healing and Graft Placement

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Recent improvements in anterior cruciate ligament (ACL) reconstruction have been notable for strategies to improve ACL healing and to improve graft placements. The controversial choice of 1-bundle or 2-bundle grafts requires an advanced knowledge of native ACL insertional anatomy and an appreciation for the kinematic effects of graft placements. Understanding the limitations of surgical techniques to place tunnels is important. Once grafts are placed, new biologic strategies to promote intra-articular and intraosseous healing are evolving. Although these biologic engineering strategies are currently experimental, they are projected for clinical application in the near future.

Keywords: ACL; ACL healing; ACL graft placement

Techniques for anterior cruciate ligament (ACL) reconstruction continue to evolve with recent notable improvements in graft placements and methodologies to improve graft healing. Particularly, the debate over 1-bundle versus 2-bundle grafts has been a catalyst to reinvestigate the anatomy of the ACL and the effects of graft placements on knee kinematics. Once grafts are placed, new biologic strategies to promote intra-articular and intraosseous healing are evolving. While some of these biologic engineering strategies are experimental, they are projected for clinical application in the near future. This review will evaluate the most recent contributions in the areas of graft placement and ACL healing.

ACL INSERTIONAL ANATOMY AND RECONSTRUCTIVE TUNNEL PLACEMENT

Background

Currently there is a debate over the need for double-bundle versus single-bundle grafts in reconstructions to restore normal kinematics to the ACL-injured knee. Advocates of the double-bundle procedure maintain that normal kinematics after ACL reconstruction are best provided by 2 grafts that essentially fill the footprints of the ACL While some clinical and laboratory data support this contention, the findings are equivocal, and most surgeons in North America still perform single-bundle reconstruction. A benefit of this debate has been a general re-evaluation of graft placements, particularly as graft placements affect knee kinematics. Central to this debate is an understanding of the natural ACLs insertional anatomy because well-placed tunnels for single-bundle and double-bundle procedures must be placed within these insertions. Ultimately, it may be fortuitous that single-bundle ACL reconstructions will be improved secondary to anatomic and kinematic insights gleaned from double-bundle procedures.

The Femoral Insertion

Girgis et al first established firm anatomic measurements for the femoral insertion of the ACL, and other studies have further characterized the anatomy. They measured the length of the femoral insertion as 23 mm with the posterior border 4 mm from the articular cartilage. Odensten and Gillquist measured a femoral insertion 18 mm long and 11 mm wide. Mochizuki et al measured a femoral length of 18.4 mm, a width of 9.5 mm, and a distance to the posterior articular cartilage of 4 mm (Figure 1). Consolidating these studies and emphasizing the more recent data, the ACL femoral insertion has an approximate average length of 18 mm, width of 10 mm, and a separation of up to 4 mm from the articular cartilage.

The long axis of the femoral insertion is rotated in the sagittal plane relative to the axis of the femur, reflecting the insertion’s congruity to the posterior border of the femoral condyle. Girgis et al measured this rotation as 25°, Odensten and Gillquist as 26°, and Heming et al as 29° (Figure 1). A generalization would be a rotation of the footprint or “flexion” of 25° to 30° relative to the femur in the sagittal plane.
The femoral insertion can also be arbitrarily partitioned into insertion sites for the anteromedial and the posterolateral bands. This delineation of the ACL into 2 bands is based on the ligament's gross appearance, best visualized when the knee is flexed 90°. The 2 bands take their names from their tibial insertions, but they can be traced to continuous origins on the femur. Both Mochizuki et al and Colombet et al described a transverse partition of the femoral insertion to delineate the attachments of the slightly larger proximal anteromedial band and the slightly smaller distal posterolateral band.

While length, width, and posterior condylar offset measurements are important, it is the proximal-distal and medial-lateral ACL locations that are most challenging to identify during surgery. The arthroscopic image of the femoral insertion changes with flexion of the knee. The anteromedial band is at the top of the notch, regardless of knee flexion angle, but the posterolateral band appears to rotate anteriorly and laterally with knee flexion. Adding to the complexity is the variable inclination of the intercondylar roof. Front-to-back visualization of the notch is best when the roof is parallel to the tibial plateau, and on average, this is when the knee is flexed 55°, but there is considerable variability. Further complexity is provided by inconsistency in the shape of the arch of the intercondylar notch. In some knees, it can be very challenging to identify and place an anatomically correct femoral tunnel.

To identify the proximal-distal position of a femoral tunnel or ACL insertion, there has been a general convention of characterizing the femoral position as referenced to the face of a clock. Although this method can be useful, it requires 2 stipulations to be precise. One, the flexion angle of the knee must be specified, and two, the transverse reference axis for the clock face must be established (Figure 2). Unfortunately most clock-face descriptions of the femoral ACL insertion have not made either of these stipulations. Only data from Mochizuki et al and Heming et al have stipulated knee flexion and an anatomic axis for a clock. Both reports specified knee flexion of 90° and a clock face referenced to the posterior femoral condyles. Viewed in this fashion, both reports placed the center of the femoral origin halfway between the apex of the notch and the edge of the articular cartilage at the base of the notch. When the knee is flexed 90° and the 3 to 9 o’clock axis is the lateral wall–articular cartilage junction, the proximal margin of the ACL will be at approximately 11 o’clock, and its distal ACL margin will be at approximately 10 o’clock.

The radiographic description of the femoral insertion has been generally accepted as determined by Bernard et al. In this study, radiographic markers were placed on the ACL insertion, and lateral radiographs were taken. The center of the femoral ACL insertion was referenced to the anterior-posterior length of the lateral condyle and the proximal-distal height of the notch. Viewed in this fashion, the center of the ACL was approximately 11.5% of the distance from the posterior condyle and 28.5% of the distance from the top of the notch. This information may be helpful with image-guided techniques for tunnel placement and with intraoperative fluoroscopic imaging of the femoral tunnel. Unfortunately, lateral radiographs of the knee are very hard to interpret for proximal-distal position of the femoral tunnel, and tunnel location only in the anterior-posterior direction can be clearly identified.

The Tibial Insertion

The ACL inserts on the intercondylar eminence of the tibia but does not attach to either the medial or lateral tubercles.
Recent studies have reported tibial insertion lengths of 15 to 18.5 mm and widths of 10 to 13 mm. An oval attachment with an approximate length of 18 mm and approximate width of 10 mm would be consistent with these studies (Figure 3).

Although general agreement exists on the size and shape of the tibial insertion, there is still debate over the best method to identify its anterior and posterior boundaries. Girgis et al. reported the entire insertion as anterior to the medial intercondylar tubercle and extending to the anterior horn of the lateral meniscus. Others have referenced the anterior border to anatomy not readily observed during arthroscopy or to a highly variable anterior horn of the lateral meniscus. Morgan et al. measured the distance from the anterior border of the posterior cruciate ligament (PCL) to the center of the ACL tibial insertion as 7 mm or 10 mm when the intercondylar spines were removed. This would place the posterior border of the ACL virtually adjacent to the most posterior margin of the tibial plateau. More recently, Colombet et al. and Heming et al. have referenced ACL tibial footprint measurements to the fovea on the tibial plateau containing the PCL (Figure 3). This indentation on the posterior tibial plateau has been described as the posterior fovea, retroeminence ridge, or PCL notch. Colombet et al. measured the distance from this notch to the posterior boundary of the ACL as 10 mm, while Heming et al. recorded this same measurement as approximately 6 mm (Figure 3). Colombet et al placed the center of the tibial insertion 19 mm anterior to the PCL notch, while Heming et al placed the center 15 mm anterior to the PCL notch. The implication of these studies is that ACL reconstructions seeking a tunnel in the center of the tibial insertion should direct a guide pin approximately 10 mm further anterior than suggested by Morgan et al. Grafts tend to lie in the posterolateral region of a tibial tunnel; therefore, tunnels should be placed slightly anterior and medial to the desired location of the graft.

Radiographically, the center of the tibial insertion in the sagittal plane has been described relative to a line parallel to the tibial plateau and passing through the most anterior and posterior points on the plateau. Amis and Jakob reported the center of the ACL tibial insertion as 43% along this line from its anterior border. Other reported measurements have been in close agreement. Image-guided surgical guidance systems use radiographic anterior-posterior and medial-lateral measurements in their algorithms to identify the location of the tibial tunnel. A practical landmark for the medial to lateral insertion of the ACL is the space between the spines of the intercondylar eminence (Figure 4). The ACL does not attach directly to these spines, but its insertion is framed on its medial and lateral aspects by these spines.

Double-Bundle Reconstruction and ACL Anatomy

A challenge to the methodology of double-bundle ACL reconstruction is the placement of 2 tunnels in the femoral and tibial footprints. On the femoral side, 2 tunnels can be placed in the long axis of the femoral footprint to approximate the 2-bundle anatomy, but on the tibial side, the 2 bundles, named for their relative insertions, are separated by a line oblique to the long axis of the insertion. Size constraints and the use of reamed tunnels can limit the ability to re-create the mediolateral relationship of the 2 bundles on the tibia. Takahashi et al. located the center of the anteromedial bundle approximately 2 mm anterior to the center of the posterolateral bundle, and Colombet et al. reported an 8-mm anterior-posterior separation of the 2 bundles' central points. Colombet et al also documented considerable variability in the obliquity of the line separating the 2 bundles on the tibia.

Another challenge in ACL reconstruction is the limitation of placing cylindrical grafts to replace a structure that is hourglass in appearance. Harner et al. reported insertion areas up to 3.5 times larger than the midsubstance area of the ligament. If the goal is an anatomic reconstruction, this is virtually impossible unless cylindrical tendon grafts will remodel to the hourglass shape of the ACL.
Kinematics of Tunnel Placement in ACL Reconstruction

Intra-articular ACL reconstruction became popular in the 1970s as a reliable procedure to eliminate the pivot-shift phenomenon. Clancy et al., early advocates of intra-articular reconstruction, recommended tunnel placements to locate the graft in the center of the tibial and femoral footprints. This was based on a concern that misplaced grafts would fail by stretching out or by limiting knee motion. Subsequent research on graft placements focused primarily on finding insertion sites that would limit graft excursions and thereby result in grafts that were more isometric. Isometricity was found to be more dependent on the femoral tunnel than the tibial tunnel, and a femoral tunnel high in the notch was most isometric.

Howell et al. subsequently identified the phenomenon of graft impingement as a cause for surgical failure. Graft impingement occurred when tunnel locations placed the graft too anterior, resulting in graft abrasion on the roof of the intercondylar notch with knee extension. Anterior graft placements on the tibia and femur have been shown to predispose the graft to failure secondary to graft elongation and loss of extension and flexion. Avoidance of graft impingement continues to be a concern, particularly as it affects the tibial tunnel.

A relatively new focus in ACL surgery has been the assessment of graft placements as they affect the kinematics of the reconstructed knee. Aside from studies documenting the perils of graft impingement, there have been surprisingly few investigations on graft placements and resulting knee kinematics. There has been a concern that knee kinematics after ACL reconstruction, particularly in the transverse plane, may not be normal after ACL reconstruction. The in vivo observation that ACL-reconstructed knees with apparently normal anterior translation still function abnormally with transverse rotation has contributed to this concern.

Cadaveric Studies

Woo et al. used a robotic system to measure anterior translation with a posterior tibial tunnel and a femoral tunnel placed via the transtibial drilling technique. This technique tends to place the femoral tunnel high in the notch in the proximal femoral footprint. Woo et al found patellar tendon grafts perform slightly better than the hamstring tendon grafts, but neither graft could re-create a normal restraint to anterior translation.

From the same laboratory, Loh et al evaluated anterior translation with a tibial tunnel in the center of the tibial footprint and a femoral tunnel placed proximal or distal in the femoral footprint. Patellar tendon grafts were used. At the critical flexion angle of 30°, where functional instability is encountered, both femoral tunnel placements provided a normal restraint to anterior translation with anterior force but not with a valgus–internal rotation torque. The distal femoral tunnel provided slightly better restraint to anterior translation when a valgus–internal rotation torque was applied.

Also from the same laboratory, Yagi et al. evaluated 1-bundle and 2-bundle grafts with 1 posterior tibial tunnel and femoral tunnels placed proximal and distal. One-bundle hamstring grafts placed in the proximal femoral tunnel were compared with split hamstring tendon grafts placed in both femoral tunnels. At 30° of flexion, neither construct could re-create a normal restraint to anterior translation, but the 2-bundle reconstruction had 2 mm less anterior translation under valgus–internal rotation torque, which was statistically significant (P < .05).

Yamamoto et al performed essentially the same experiment except a lower femoral tunnel was used for the 1-bundle reconstructions. They reported normal anterior translations at 30° of flexion for single- and double-bundle reconstructions under both loading conditions. At 90° of flexion, the double-bundle reconstruction provided slightly better anterior translation.

Scopp et al. tested a 1-bundle reconstruction with a posterior tibial tunnel and either a proximal or distal femoral tunnel. At 30° of flexion, neither proximal nor distal femoral tunnels provided normal anterior translation with anterior force. However, with internal rotation torque, grafts placed in the distal femoral tunnel provided a better restraint to internal rotation.

T. C. Battaglia et al. (unpublished data, 2006) compared single-bundle ACL reconstructions with 2 separate tunnel combinations. One graft placement was with the traditional transtibial technique with the tibial tunnel posterior and the femoral tunnel proximal. The other graft placement had the tibial tunnel central in the tibial footprint and the femoral tunnel central in the femoral footprint. Grafts centralized in the footprints restored normal anterior translation and internal rotation, while grafts placed by the transtibial technique did not.

In summary, the in vitro biomechanical studies have demonstrated the shortcomings of tunnels placed by the transtibial technique. This technique places the tibial tunnel in the posterior tibial footprint and places the femoral tunnel in the proximal femoral footprint. Laboratory tests of ACL reconstructions using these tunnel positions have not been shown to re-create a normal restraint to anterior translation or internal rotation. The studies also documented a preferred location of the femoral tunnel in the mid to distal femoral footprint. If a single-bundle graft is placed in the central femoral footprint, the resulting knee kinematics mirror those of a 2-bundle graft except at 90° of flexion.

Clinical Studies

There are a limited number of clinical studies evaluating the effect of tunnel position on knee kinematics. Most of these studies have focused on graft impingement as a cause for failure and not knee kinematics.

Radiographs have been used to identify tunnel position, but there are technical limitations because on the femoral side, only the anterior-to-posterior tunnel position can be identified and not the proximal-to-distal location.

Muneta et al. identified a tendency for knees with tibial tunnels placed too anterior or lateral to have greater...
anterior laxity and synovitis, presumably from impingement on the roof and lateral wall of the intercondylar notch. Khalfayan et al\(^4\) performed a similar analysis and also found greater anterior translation when the tibial or femoral tunnels were placed too anterior.

Sommer et al\(^5\) reviewed femoral tunnels in knees undergoing revision surgery and identified femoral tunnels placed too anterior and grafts placed too superior in the notch as causes for failure. Howell et al\(^6\) indirectly reinforced the concern with grafts placed too high in the notch by recording the angle of tibial tunnels on anterior radiographs. In the Howell study, the femoral tunnels were drilled endoscopically so the angle of the tibial tunnel indicated the location of the femoral tunnel. A vertical tibial tunnel predisposed the knee to greater anterior laxity and loss of flexion. A more oblique tibial tunnel resulted in normal laxity and a better range of motion. Because a vertical tibial tunnel will place a femoral tunnel high in the notch and a more oblique tibial tunnel will place a femoral tunnel lower in the notch, the implication was that a femoral tunnel in the distal femoral footprint would produce better results.

Several studies have compared the results of transtibial versus 2-incision ACL reconstruction.\(^4\),\(^8\) These studies have questioned whether the femoral tunnel drilling method would affect clinical results. However, these studies describe surgical techniques that placed femoral tunnels in the same position regardless of drilling technique. While radiographs revealed a greater femoral tunnel obliquity with the 2-incision technique, the femoral aperture was not different between the 2 techniques, and results were similar.\(^3\),\(^4\),\(^8\)

In summary, clinical studies have documented a deterioration of results if the tibial or femoral tunnels are placed too anterior secondary to impingement. Limited data have supported placement of the femoral tunnel lower in the notch to promote survival of the graft and normal laxity.

**Surgical Strategies for Tunnel Placement**

Placement of the tibial tunnel entails deciding where to start on the tibial cortex and where to exit on the tibial plateau. If the surgeon is using the transtibial technique to drill the femoral tunnel, then the choice of these 2 points is constrained by the concern for the femoral tunnel.\(^4\) If the femoral tunnel is drilled outside-in or through an anteromedial portal, then there is greater freedom in choosing a starting point.

The exit point on the tibial plateau is chosen to avoid impingement. However, a horizontal graft will generally resist anterior forces better than a vertical graft; therefore, a goal is to place the tibial tunnel central in the footprint, provided impingement does not occur. A reasonable goal is to place the tibial tunnel in the center of the tibial footprint. Impingement can be checked by extending the knee and visualizing the relationship between the notch and the proposed tunnel location.

A tunnel centered in the tibial ACL insertion would be 7 mm anterior to the PCL notch based on measurements by Heming et al,\(^4\) or 19 mm anterior to the PCL notch based on measurements by Colombet et al,\(^2\) or 19 mm anterior to the PCL notch based on measurements of the PCL notch of the anterior cruciate ligament are represented by black ellipses. The quadrilateral space is bordered by the medial collateral ligament (MCL), pes anserinus, medial border of the tibial tubercle, and the tibial articular surface. Recommendations for drilling a tibial tunnel are generally referenced to the borders of this space.

Figure 5. The quadrilateral space on the anteromedial tibial where tibial tunnels are often initiated is outlined, and the footprints of the anterior cruciate ligament are represented by black ellipses. The quadrilateral space is bordered by the medial collateral ligament (MCL), pes anserinus, medial border of the tibial tubercle, and the tibial articular surface. Recommendations for drilling a tibial tunnel are generally referenced to the borders of this space.

PCL notch based on measurements by Colombet et al.\(^2\) The recommendation of Morgan et al, based on a transtibial drilling method, may place the tibial tunnel too posterior.\(^4\),\(^8\),\(^11\) Based on recent measurements, the center of the tunnel should be approximately 15 mm anterior to the PCL notch. Other landmarks are helpful, specifically the intercondylar tubercles that lie on each side of the ACL. The tunnel may be drilled slightly eccentrically in the anteromedial direction because a graft will displace to the posterior and lateral aspects of the tunnel.

To avoid impingement, a drill guide is available that places the tibial tunnel posterior to the intercondylar notch of the femur when the knee is extended.\(^4\) The guide may place tunnels posterior on the tibia, and impingement of the graft on the PCL can result. A medial transtibial tunnel starting point that sometimes originates in the medial collateral ligament (MCL) has been recommended with this guide.\(^4\) A lower femoral tunnel is also an effective strategy to prevent ACL graft impingement.

If the transtibial drilling method is used, the starting point for the tibial tunnel must be chosen carefully to drill the femoral tunnel (Figure 5). Chhabra et al\(^5\) recommended starting halfway between the tibial tubercle and the posteromedial tibia and setting the drill guide angle 7° greater than the tendon length of a patellar tendon graft. This approximation can be modified based on the placement of the anteromedial portal. Morgan et al\(^6\) recommended starting 1 cm superior to the pes anserinus and 1.5 cm medial to the tibial tubercle and directing the tunnel 7 mm anterior to the PCL to re-create the coronal angle of the ACL (Figure 5). Recently Howell et al\(^6\) reported intentionally drilling through the fibers of the MCL to create an angle...
between the tunnel and the tibial plateau of less than 75° in the coronal plane. Heming et al\textsuperscript{42} documented in cadaveric knees the starting point for a guide pin to traverse the central tibial and femoral ACL insertions. The point was closer to the articular surface than the pes anserinus and closer to the tibial tubercle than the MCL. This starting point would result in a short tibial tunnel (<30 mm), which might present a problem for fixation and healing. Arnold et al\textsuperscript{5} performed an interesting study to document the challenge of transtibial drilling. In the study, a tibial tunnel started at the corner of the pes anserinus and MCL and was directed to the posterior tibial footprint. The resulting femoral tunnel drilled by the transtibial technique was extremely proximal in the notch and often completely missed the femoral footprint.

Because of concerns with the transtibial drilling method, there has been renewed interest in alternatives for femoral tunnel drilling\textsuperscript{35,79} A common alternative is to drill the femoral tunnel through an anteromedial portal. However, this requires knee flexion of approximately 120° to avoid injury to the posterolateral structures and can be challenging when a leg holder is used. Techniques to avoid neurovascular injury include keeping the foot up to allow maximum knee flexion, drilling through a low auxiliary anteromedial portal, and visually checking that the guide pin will exit anterior to the neurovascular structures. Other alternatives include drilling outside-in through a second incision or using custom reamers. Flexible reamers have been introduced that permit drilling through an anteromedial portal without extreme knee flexion. Also, reamers have been introduced where a guide pin is drilled outside-in and a reaming tip is attached to the pin intra-articularly.

Strategies for placement of the femoral tunnel have been most problematic for a host of reasons. Foremost is the difficulty referencing known anatomic landmarks to the ACL femoral attachment. The practice of referencing points on the intercondylar notch to the face of a clock is common but not accurate unless the horizontal axis of the clock and the flexion angle of the knee are both stipulated. A confounding variable is the inclination of the intercondylar roof in the sagittal plane.\textsuperscript{43} Generally, the best visualization of the notch is provided when the knee is flexed 50° to 60° to place the roof of the notch parallel to the tibial plateau. Yet the femoral tunnel is drilled when the knee is flexed approximately 90° for the transtibial method and approximately 120° for the anteromedial portal drilling method. Extrapolating from the studies by Heming et al\textsuperscript{42} and Mochizuka et al,\textsuperscript{67} the central point of the ACL femoral insertion is located halfway between the apex of the notch and the base of the notch (femoral articular margin) when the knee is flexed 90° (Figure 2). A guide pin can be placed through an anteromedial portal to reach this point and drilled safely out the lateral femur when the knee is flexed over 120°. It may be possible with the transtibial method to produce a tunnel in this position, but the corresponding tibial tunnel must start on the medial tibia, often within the fibers of the MCL, and exit in the posterior region of the tibial footprint.

Technique articles for double-bundle ACL reconstruction have recommended stacking 2 tibial tunnels anterior to posterior with a 2-mm separation between them.\textsuperscript{1,15,34,67,69,116} The posterior tunnel has been centered 5 mm anterior to the PCL. There has been an attempt to place the anterior tunnel slightly medial and the posterior tunnel slightly lateral in recognition of the anatomy where the 2 bundles insert mediolateral and anterior-posterior to each other. The 2 femoral tunnels can be placed directly if fibers of the original ACL can be identified. However, in chronic cases, the insertion is less discernable, and guidelines are necessary for placement of the tunnels. Yasuda et al\textsuperscript{116} recommended a method for identifying the center of the posterolateral bundle by flexing the knee 90° and locating the femur-tibial articulation point. They placed the center of the posterolateral bundle 5 to 8 mm superior to this point and recommended placing the anteromedial tunnel proximally. The posterior margin of the femoral tunnel should be within a few millimeters of the posterior condyle articular cartilage margin. The so-called resident’s ridge in the intercondylar notch is a good anatomic border for the anterior border of the ACL footprint.

Image-guided navigation systems are available to place tunnels in ACL reconstruction. These systems use algorithms derived from imaging and anatomic studies to place tunnels in the anatomic centers of the ACL insertions.\textsuperscript{12} There has been an observation that such tunnel placements tend to be more anterior on the tibia and lower in the femoral notch than tunnels placed with conventional drill guides (T. C. Battaglia et al, unpublished data, 2006). If clinical results can be improved with image-guided technology, then the added time and invasiveness of the technology will be warranted.

Summary of ACL Insertional Anatomy and Reconstructive Tunnel Placement

There has been a renewed interest in the insertional anatomy of the ACL because of concerns with the kinematics of reconstructed knees. Cadaveric studies have found that grafts centered in the ACL footprints provide a better restraint to anterior translation than grafts placed vertically. Whether acceptable kinematics requires 1-bundle or 2-bundle grafts has not been resolved. Acceptable graft placements require an understanding of the shortcomings of the transtibial drilling method, and the use of drilling strategies other than the transtibial method may be necessary.

NATIVE ACL HEALING

In humans, the ACL fails to heal after rupture. Even with primary repair, the ACL still fails to heal in the majority of patients,\textsuperscript{92,95,107} although carefully selected patients may have a good clinical outcome with primary repairs. This makes the ACL vastly different from other ligaments in the body, including the MCL, which heals readily with functional bracing.
The cause of the failure of the ACL to heal has been a matter of some debate. Much research has focused on differences in cell behaviors, suggesting that uniquely disabled fibroblasts populate the ACL. The results of these studies have suggested that the failure of healing is due to the failure of the cells and blood vessels within and around the ACL to mount an adequate healing response. A second school of thought has more recently evolved based on observations of the human ACL after rupture. In this group of papers, the human ACL was found to actually have a proliferative vascular and neurogenic response to rupture, a response similar to that found in the injured MCL. In addition, there was noted to be no bridging of the gap between the ruptured ends of the ACL. Most connective tissues heal by filling the wound gap with a fibrin-platelet clot or provisional scaffold; however, this was missing in the ACL wound site. Thus a second hypothesis for ACL healing failure was proposed—that the failure of the ACL to heal was a result of a lack of wound-site filling (Figure 6).

The persistence of an unfilled gap in the wound site of the ACL is not unique to the ligament but is seen in meniscus, cartilage, and rotator cuff tendons, all tissues within the intra-articular environment. All of these tissues have lower success rates of clinical and histologic healing when compared with tissues found outside of the joint where a fibrin-platelet clot fills the wound site. This may be because of the intra-articular environment of a synovial joint where blood does not form a clot but rather disperses throughout the joint as a hemarthrosis. This process is helped by increased levels of "clot-busting" enzymes that appear in the joint fluid shortly after trauma. The prevention of clot formation within the joint is likely helpful in minimizing joint fibrosis after an injury, thus preserving motion within the joint and maintaining short-term function at the expense of tissue healing and long-term joint function.

Because of the clinical importance of the ACL and ACL injury, much interest has focused on enhancing healing of the ACL. Work has focused both on improving the cellular and vascular response, as well as providing substitute scaffolds in the wound site. The results of these studies are outlined in the following sections.

Figure 6. Platelet-derived growth factor (PDGF)-AB expression within ligament defects 7 days after injury. On the left, the medial collateral ligament (MCL) defect has filled with tissue that is releasing PDGF-AB, while on the right, the untreated anterior cruciate ligament (ACL) defect remains empty. Immunohistochemistry for PDGF-AB, where a red stain is positive. 100× magnification.

**Cellular and Vascular Healing Response of the ACL**

Growth factors, such as transforming growth factor beta (TGF-β), platelet-derived growth factor (S. Kawamura et al, unpublished data, 2004), and fibroblast growth factor (FGF) have been used to stimulate ACL cell proliferation, collagen production, and cell migration with promising results in vitro. Based on these results in the laboratory with cells, healing of the ACL using 1 or 2 growth factors placed in an ACL defect has been tried but with limited success that would be clinically relevant. For example, in a study of MCL transection in rabbits, Spindler et al reported an increase in scar size, but not scar strength, with the addition of TGF-β. Kobayashi et al noted improved filling and vascularity surrounding a central defect of the ACL in a canine model with implantation of a basic FGF pellet; however, no biomechanical testing was performed in that study. The limited success of the use of 1 growth factor to stimulate ligament healing in a clinically important way has led to the search for alternative approaches, including use of scaffolds loaded with multiple growth factors and extracellular matrix molecules.

**Substitute Scaffolds to Fill the Wound Site**

If we accept that premature loss of filling of the wound site may be partly responsible for the failure of the ACL to heal, then it logically follows that filling the wound site in vivo with an appropriate substitute for clot may lead to improved healing. A historical approach was to augment a primary ACL repair using a synthetic device to protect the sutured ACL from early stress. The Kennedy Ligament Augmentation Device (LAD) was used in this fashion as a nonabsorbable braid of polypropylene. However, comparative studies found reconstructions with autologous grafts functioned better than attempted repairs supplemented by an LAD. Furthermore, complications of synovitis and effusion have been associated with the LAD, and use of this device has been abandoned.

The best wound filler would adhere to the wound edges and provide a bridge across the wound site for cells to invade and remodel. It would encourage the cells to deposit extracellular matrix proteins and subsequently strengthen the tissue within the wound with time. The biologic nature of the repair would also ideally support repair of small amounts of damage. Different candidate designs for such scaffolds have been tested in animals where both partial and complete ACL defects have been made and substitute clots or scaffolds introduced into the wound sites. The histologic and biomechanical results are detailed below for hyaluronic acid and collagen-platelet hydrogels.

**Hyaluronic Acid**

Intra-articular injections of hyaluronic acid have been used to stimulate healing of a central defect rabbit model, and histologic improvements of wound covering, vascular response, and collagen production were noted in the ligaments treated with hyaluronic acid. However, no biomechanical testing was reported, and no follow-up studies have been published.
Collagen-Platelet Hydrogels

Platelets are key cellular mediators of wound healing. Once they are concentrated in a wound site within a blood clot, they release over 20 known cytokines in a sequential fashion and recruit other cells, including white blood cells and fibroblasts, to help with the wound repair process. Collagen-platelet hydrogels take advantage of this complex release system and use platelets to deliver growth factors to the wound site.

Recent work has used these materials to stimulate healing in a central defect of a canine ACL and in a complete transection of a porcine ACL. The collagen is critical to this system because it stimulates platelets and it requires a matrix metalloproteinase cofactor to be degraded.

Collagen-Platelet Hydrogels to Stimulate Healing of Central ACL Defects. In 1 recent study, a collagen-platelet gel was used to treat a central ACL defect in a previously established in vivo nonunion model. Histologically, the untreated ACL defects had a persistent gap, even at the 6-week time point. In contrast, the ACL defects treated with a stabilized provisional scaffold had filling of the defect similar to the extra-articular patellar tendon control (Figure 7). However, the treated ACL had only partial defect filling, whereas the patellar tendon defects had almost complete filling of the injury site with repair tissue. Biomechanically, the treated ACL defects had a 40% increase in strength at 6 weeks, which was significantly higher than the 14% increase in strength previously reported for untreated defects for the same time period ($P = .03$). This study concluded that the use of a substitute bridge could stimulate healing of a central ACL defect.

Collagen-Platelet Hydrogels to Stimulate Healing of a Complete ACL Transection. Although the results in the central defect model were promising, patients do not suffer central defects but rather total ruptures. In an animal model, collagen-platelet scaffolds have recently been used to enhance healing of complete transections of the ACL treated with suture repair (Figure 8). In a study of bilateral ACL transactions, 1 side was treated with suture repair alone, while the other side was treated with suture repair supplemented with a collagen–platelet-rich plasma (PRP) hydrogel. The animals were allowed to bear weight immediately postoperatively, and no immobilization was used. The supplementation of suture repair with a collagen-PRP hydrogel resulted in significant improvements in load at yield, maximum load, and linear stiffness after 4 weeks. While these preliminary results suggest primary repair supplemented with a growth factor gel may be useful in selected patients at some point in the future, these results are still very preliminary, and much additional work is required before this new approach can be considered for clinical use.

Summary of Native ACL Healing

One of the most challenging and important problems facing orthopaedics today is the failure of tissues within the joint, including the ACL, to heal after injury and surgical
New Concepts in Tendon-to-Bone Healing

The biology of healing between the grafted tendon and bone remains incompletely understood. The biologic and biomechanical environment in the bone tunnel results in formation of an attachment site that differs from the native ligament-bone insertion. Experimental studies have found that tendon healing in a bone tunnel begins by reactive fibrovascular tissue (scar) formation, followed by bone ingrowth into the tendon-bone interface. The overall structure, composition, and organization of a normal insertion site do not regenerate. The poor healing response likely relates to insufficient expression of genes that direct formation of the complex structure and composition of the insertion site.

Current work suggests that 3 fundamental factors are responsible for the lack of appropriate molecular signals (gene expression) and cell differentiation resulting in the ineffective healing response between tendon and bone. These factors are (1) the presence of inflammation in the postnatal organism, (2) tendon-bone interface motion, and (3) an insufficient number of undifferentiated cells at the healing tendon-bone interface. These factors will be considered in turn. Other biologic factors that play a role in tendon-to-bone healing will also be discussed.

Inflammation in Tendon-to-Bone Healing. Healing after surgery or trauma begins with an influx of inflammatory cells. Important lessons about inflammation, tissue healing, and regeneration can be learned from the study of fetal development and fetal wound healing. Wounds in the embryo and early fetus are known to heal by tissue regeneration, in a process that has been termed “scarless healing.” The absence of a significant inflammatory response in fetal wounds is likely to be an important factor in scarless healing. In contrast, inflammation plays a critical role in wound healing in the postnatal organism. The transition stage after which inflammatory cells (such as macrophages) accumulate in a fetal wound is coincident with the stage in mouse fetal development after which wounds heal by scar.
This information indicates that inflammation, while essential for efficient tissue repair in adults, leads to healing by scar tissue rather than regeneration of phenotypically normal tissue. The rapid influx of inflammatory cells after tendon transplantation in a bone tunnel likely results in cellular and molecular signals that ultimately lead to fibrosis rather than tissue regeneration. Furthermore, the normal inflammatory response that occurs after surgery is likely prolonged by tendon-bone interface motion.

The Role of Macrophages in Tendon Healing in a Bone Tunnel. Because macrophages play a critical role in the adult inflammatory response to wounding, macrophage accumulation around the tendon graft in the bone tunnel in a rat ACL reconstruction model has been examined. It was hypothesized that macrophage depletion may lead to regeneration of a normal insertion site in a form of scarless healing rather than reactive scar tissue healing. The hypothesis was tested by depleting macrophages in a rat ACL reconstruction model using weekly intraperitoneal injections of liposomal clodronate (a bisphosphonate that selectively induces macrophage apoptosis).

Macrophage-depleted animals exhibited accelerated tendon-bone healing compared with untreated control specimens as demonstrated by significantly reduced fibrovascular “scar” tissue formation in the tendon-bone interface, enhanced bone ingrowth into tendon, and improved collagen fiber continuity between tendon and bone. These structural changes were accompanied by a significant improvement in load-to-graft failure and graft stiffness compared to controls. Taken together with the finding that scarless healing coincides with the absence of functioning macrophages, these data suggest that macrophages and other inflammatory cells may inhibit expression of tissue-specific patterning genes that direct formation of the insertion site. Techniques to modulate inflammation and macrophage accumulation may improve tendon-bone healing and may enhance the structural integrity of the healing tendon attachment site. Unfortunately non-steroidal anti-inflammatory medications have too broad an effect on inflammation and healing, and it has been well documented that these medications often delay ligament healing.

The Role of Graft-Tunnel Motion in Tendon-to-Bone Healing. Relative graft-tunnel micromotion may contribute to sustained inflammation due to repetitive “microinjury” at the healing interface. The effect of graft-tunnel motion on tendon-to-bone healing was evaluated using a rabbit ACL reconstruction model. It was found that graft-tunnel motion was greatest at the tunnel apertures and least at the tunnel exit.

Also, bilateral ACL reconstructions were performed in rabbits, and histomorphometry was used to compare tendon-bone healing between the intra-articular tunnel aperture, mid-tunnel, and tunnel exit. Healing of the graft was found to be slowest at the intra-articular tunnel apertures, as evidenced by a wider scar tissue interface between tendon and bone. The width of the tendon-bone interface was greater at the intra-articular aperture than the tunnel exit in the femoral tunnel (P = .04), and there was an inverse correlation between motion and healing in the femoral tunnel (P = .005). Osteoclasts were preferentially found at the tunnel apertures. Graft-tunnel motion may impair early graft incorporation and may lead to osteoclast-mediated bone resorption. These findings also support graft-tunnel motion as a cause of “tunnel widening.”

Further support for the concept that graft-tunnel motion can impair healing was provided by Sakai et al. These investigators used a rabbit ACL reconstruction model and compared various periods of immobilization up to 6 weeks. The load to failure was higher in the immobilized animals, and histologic examination found closer apposition of tendon to bone in the immobilized animals. These authors concluded that immediate motion delayed graft incorporation in the bone tunnel after ACL reconstruction.

A clinical study also provides support for the effect of graft-tunnel motion on graft incorporation. Yu and Paessler compared 33 patients who underwent ACL reconstruction with a soft-tissue graft followed by “aggressive rehabilitation” and 32 patients who underwent the same procedure combined with meniscal repair or microfracture who had “conservative rehabilitation.” They reported greater tibial tunnel widening in the aggressive rehabilitation group. These findings support graft-tunnel motion as a cause of tunnel widening and show that the mechanical environment including possibly hydrostatic pressure within the bone tunnel can influence graft healing. Further information is required to understand how the magnitude, timing, and type of mechanical loading at the graft-bone interface influence graft incorporation.

Stem Cells in Tendon-to-Bone Healing. A sufficient population of undifferentiated cells (“stem cells”) is likely required for optimal tissue regeneration. Abundant stem cells are present during fetal tissue development, and fewer undifferentiated cells are present during healing in the postnatal organism. Several studies have tested the effect of local stem cell delivery on tendon-to-bone healing. Lim et al. performed bilateral ACL reconstructions in an animal model with grafts coated with mesenchymal stem cells (MSC) in a fibrin glue in 1 limb and fibrin glue only in the other limb. The control reconstructions healed by formation of a fibrous tissue interface between tendon and bone, while the MSC-treated grafts had cartilage at the tendon-bone interface. The interface stained positively for type II collagen in the MSC-treated grafts and was more similar to a native direct ligament insertion. At 8 weeks, the MSC-treated grafts had significantly higher failure load and stiffness. Further studies are necessary to examine the fate of the transplanted cells and to understand their role in healing.

Bone Formation in ACL Graft Healing

Studies have found that bone ingrowth plays an important role in graft-to-bone fixation. Several strategies have been demonstrated to improve bone ingrowth into a tendon graft placed in a bone tunnel. Most of these have involved the use of osteoinductive cytokines such as bone morphogenetic proteins (BMPs). Recombinant human BMP-2 (rhBMP-2) in an injectable calcium phosphate matrix has been placed in a bone tunnel in a rabbit ACL reconstruction model. The investigators found that rhBMP-2 treatment
led to a significant increase in the width of new bone formation at the tendon-bone interface in a dose-dependent fashion. The addition of rhBMP-2 resulted in a narrower fibrous tissue interface between tendon and bone. Tunnel diameters in the rhBMP-2 group were significantly smaller (15%-45%) than the carrier group. The rhBMP-2 group demonstrated significantly increased stiffness at 8 weeks.

Martinek et al used gene therapy techniques to induce endogenous synthesis of BMP-2 in the healing tendon-bone interface in a rabbit ACL reconstruction model. Semitendinosus grafts were infected in vitro with adenovirus-BMP-2 (AdBMP-2). Untreated grafts served as controls. They found formation of a fibrocartilage interface between tendon and bone in the experimental group, in contrast to a fibrous tissue insertion in the control group. The stiffness and the ultimate load to failure were significantly higher in the specimens with an AdBMP-2-transduced graft when compared with the controls at 8 weeks ($P < .05$).

Bone morphogenetic protein-7 (also known as osteogenic protein-1) has similar osteoinductive activity in the bone tunnel. Mihelic et al. used BMP-7 in a sheep ACL reconstruction model and reported improved bone formation at the tendon-bone interface, with significantly higher load to failure in the BMP-7-treated animals at both 3 and 6 weeks ($P < .01$).

Other modalities that can improve bone formation include low-intensity pulsed ultrasound. Walsh et al. performed ACL reconstruction in a sheep model using a free tendon graft. Low-intensity pulsed ultrasound (LIPUS) was delivered daily to the lateral aspect of the femur and the anteromedial tibia, while control animals received no treatments. Histologic grading of the healing tendon-bone insertion site showed distinct differences between groups. There was a marked increase in vascularity in the LIPUS-treated animals with increased expression of vascular endothelial growth factor. There was improved new bone formation at the healing tendon-bone interface in the LIPUS-treated animals.

Further support for the role of osteoinduction in graft healing is provided by studies that have used periosteum wrapped around the tendon graft in the bone tunnel. Chen et al. and Ohtera et al. independently have used a rabbit model in which the long digital extensor tendon was transplanted in a bone tunnel in the proximal tibia. Both studies found improved bone and fibrocartilage formation in the periosteum-treated animals compared with controls. Chen et al. reported a significant increase in graft attachment strength at 8 and 12 weeks. Ohtera et al. compared fresh and frozen periosteum and found superior biomechanical and histologic results at 4 weeks using fresh periosteum, suggesting that bioactive factors in the periostea were responsible for the improved results.

Osteoconductive materials may also play a role in improving tendon healing in a bone tunnel. Tien et al. used calcium phosphate cement (CPC) in the femoral tunnel in a rabbit ACL reconstruction model using a semitendinosus tendon. There was markedly improved bone formation in the CPC-treated animals, with significantly greater ($P < .05$) tensile strength at 1 and 2 weeks postoperatively.

The Role of Bone Resorption in ACL Graft Healing

A common clinical concern after ACL reconstruction is radiographic expansion of the bone tunnels. The causes and clinical relevance of this phenomenon remain unclear. Possible causes for tunnel widening include biologic factors (inflammatory mediators and synovial fluid cytokines) and mechanical factors (graft-tunnel micromotion). Some authors have also considered an immune response to allograft tissue as a possible cause. The final common pathway is likely to be osteoclast-mediated bone resorption. In clinical practice, bone loss due to tunnel expansion may compromise and complicate revision surgery. A recent study demonstrated that increased knee laxity correlated with radiographic femoral tunnel widening in ACL reconstructions using hamstring tendon.

The role of osteoclastic bone resorption in tendon-to-bone healing in a rabbit ACL reconstruction model has been evaluated by modulating osteoclastic activity using receptor proteins and evaluating the bone tunnels at 2, 4, and 8 weeks. Inhibition of osteoclasts resulted in a greater amount of bone surrounding the tendon at all time points ($P < .05$) and increased stiffness at 8 weeks ($P = .04$). These results demonstrate that inhibition of excessive osteoclastic activity may improve tendon-to-bone healing.

The Role of Vascularity in Tendon-to-Bone Healing

Although it is well known that adequate vascularity is necessary for efficient connective tissue healing, it is not known if strategies to increase local vascularity at the healing tendon-graft attachment site can improve graft-to-bone healing. A recent study examined the effect of vascular endothelial growth factor (VEGF), a potent mediator of angiogenesis, on graft healing in a sheep model. There was increased vascularity and cellularity in the VEGF-treated grafts, but their stiffness was lower and laxity greater than controls ($P = .017$). These preliminary data suggest that excessive vascularity may have detrimental effects on the healing ACL graft.

Matrix Metalloproteinases in ACL Graft Healing

Matrix metalloproteinases (MMPs) play a central role in degradation and remodeling of the extracellular matrix during healing and graft remodeling. Yet there is little information available on the role MMPs play in tendon-to-bone healing. Demirag et al. tested an MMP inhibitor in a rabbit ACL reconstruction model. The interface tissue in the treated specimens was more mature and contained numerous perpendicular collagen bundles (Sharpey fibers). The strength was significantly greater in the MMP-inhibited specimens than in untreated controls at both 2 and 5 weeks. This study demonstrates that MMP inhibition can improve tendon graft healing in a bone tunnel.

FUTURE DIRECTIONS

The concepts discussed above suggest novel and exciting approaches to augment the basic biology of tendon-to-bone
healing. Future techniques to improve tendon-to-bone healing may include use of cytokines to provide important signals for tissue formation and differentiation, gene therapy techniques to provide prolonged presence of an important molecule for healing, the use of stem cells to provide a population of undifferentiated cells, and the use of transcription factors to direct nuclear gene expression. Commercially available systems to create a platelet-rich plasma or platelet factors to direct nuclear gene expression also hold promise. Finally, modulation of the mechanical environment may have profound effects on the cellular and molecular events at the healing tendon-bone interface.

REFERENCES


