Mechanical Testing of Ligament Fixation Devices

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Summary: The fixation of soft tissue grafts used in anterior cruciate ligament reconstruction (ACL) is a determining factor for the long-term success of the ACL reconstruction. This article provides an overview of the mechanical test methods used in our laboratory and the biomechanics literature to compare methods of soft tissue fixation that are available to the practicing orthopaedic surgeon. The basics of uniaxial tensile testing are reviewed with particular attention to the differences between the structural properties of the reconstructed graft and the material properties of the graft itself. The effects of the stiffness of individual components on the overall structural response of the system are studied in the context of a simple series spring model. Techniques used to measure graft cross-sectional area, overall elongation, and graft strain are reviewed. The importance of controlling temperature and hydration during and between tests is emphasized. Failure testing, cyclic loading, and in vitro evaluation of joint kinematics are reviewed with specific attention to factors that affect the overall test results. The final section provides a brief overview of the factors that contribute to a well-designed experiment and statistical analysis. It is hoped that the information in this article will provide the reader with the information necessary to critically evaluate the multitude of experimental studies that compare the mechanical characteristics of different ligament fixation techniques. Key Words: Anterior cruciate ligament-Soft tissue fixation-Cyclic loading.

INTRODUCTION

The decision as to which fixation device to use for ligament reconstruction has become increasingly difficult as more and more fixation devices reach the market. A large part of the market is targeted at reconstruction of the anterior cruciate ligament (ACL). The fixation devices available include staples, screw and washer, interference screws, crosspin designs, and devices for cortical fixation. Fixation in bone ranges from purely cancellous to purely cortical. Many factors influence a surgeon's choice of fixation, including price, ease of use, and efficacy for a particular type of soft tissue graft. Mechanical testing of these devices offers an objective means to compare strength and stiffness characteristics, both at the time of initial fixation using cadaveric or animal tissue, or for in vivo studies using animal models. This information is useful in the design phase and for comparison between devices, but the data also can provide the surgeon with guidance for the selection of a fixation device for a particular application. Butler identified three functions of graft fixation devices: (1) to provide apposition of the graft with surrounding tissue to allow incorporation; (2) to resist slippage or migration under repeated loading; and (3) to resist sudden traumatic loading. This article provides an overview of the types of mechanical tests that can be used to address the latter two points for the analysis and comparison of ligament fixation devices.

STRUCTURAL PROPERTIES VERSUS MATERIAL PROPERTIES

To understand the biomechanical factors that are important in evaluation of ligament fixation, it is useful to understand the means of evaluating the tensile properties of a bone-ligament-bone prepa-

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ration. Two sets of mechanical quantities can be obtained from these tests: the structural properties of the complex, as determined from the loadelongation curve, and the mechanical properties of the ligament substance, as determined from the stress-strain curve (Fig. 1). Structural properties that are commonly reported for tensile test data include the ultimate load, ultimate elongation, stiffness, and energy absorbed to failure (Fig. 1A). The ultimate load is the maximum load sustained by the complex during testing, and the ultimate elongation is the change in length at which this load occurred. Stiffness is the slope of the load-elongation curve in the linear portion. Energy absorbed to failure is the area under the load-elongation curve. For the evaluation of fixation devices, the initial failure load and initial failure elongation also are often reported. The initial failure load is the load at which the load-elongation curve deviates from linearity. or the point at which damage to the complex is first initiated. These structural properties are affected by the material properties of the soft tissue, the geometry of the soft tissue (that is, cross-sectional area), and the strength/stiffness characteristics of the attachments of the soft tissue to the bone. The latter is often the focus of studies of ligament fixation strength. Material properties describe the material characteristics of the soft-tissue substance itself, affected in part by the organization, orientation, and type of collagen, as well as interactions with other matrix constituents. Material properties include the tensile strength, ultimate strain, and tangent modulus (Fig. 1B). Stress is determined by dividing the applied tensile load by the tissue initial cross-sectional area. The tensile strength is the maximum stress resisted by the tissue, and the ultimate strain is the strain at which the tensile strength occurred. The tangent modulus refers to the slope of the stress-strain curve in the linear portion.

Experimental determination of the tensile properties of soft tissue grafts poses several unique problems. The simplest approach would be to isolate the tissue from any bony attachments for material property measurement. Clamping the ends of the tissue can cause stress risers that will cause premature failure or slipping at the clamp sites. This will prevent the determination of tensile strength and ultimate strain. To remedy these problems, a bone–ligament–bone complex is used when possible. The bone–ligament–bone preparation can provide secure clamping and better approximates



FIG. 1. (A) Structural properties as defined using the load-elongation curve. (B) Material properties as defined using the stress-strain curve.

in situ conditions, but the tensile test data obtained from such a preparation include contributions from both the hard and soft tissue properties, making it difficult to isolate the properties of the ligament substance from the insertions to bone. To address this issue, accurate measurement of ligament crosssectional area and measurement of strain in the central portion of the soft tissue are necessary to ensure that the material properties are representative of the tissue itself.²¹

Freeze clamps have been used to grip tendinous graft tissue directly. These clamps use either liquid nitrogen or dry ice to freeze the tissue within the clamped region, providing a local hardening of the tissue that can prevent failure of the tendon at the grips. Care must be taken to avoid freezing tissue outside the clamped region. Our laboratory has used this method successfully for testing ACL fixation with semitendinosus and gracilis tendon grafts.

THE WEAK LINK IN LIGAMENT FIXATION

With few exceptions, ligament reconstructions fail at or near the fixation to bone when tested in



FIG. 2. Schematic illustrating two springs of different stiffness in series under application of a tensile load F.

tension. Depending on the device, the failure mode can be a suture knot, slipping of sutures through the soft tissue graft, cracking of a bone block (for bone plug fixation), slipping of an interference screw, slipping of soft tissue past an interference screw, or several other variations. The amount of slipping or compliance usually is much greater at the fixation site or in materials used to attach the soft tissue to the fixation site than is compliance of the soft tissue graft itself. One possible exception for ACL reconstruction would be interference screw fixation of patellar tendon grafts.

The relationship between different tensile elements in series such as a tendon and a suture can be studied by using the analogy of two springs in series under tension (Fig. 2). If a tensile force F is applied to the system, the total elongation is $U = u_1 + u_2$, where u_1 and u_2 are the elongations of each spring. Thus, it is clear that if the first spring is much stiffer than the second, that is, $k_1 >> k_2$, then the total measured elongation will be dominated by the elongation u_2 of the spring with stiffness k_2 . Furthermore, the total force F in the system is related to the overall system stiffness K and displacement U through

$$F = KU = K(u_1 + u_2).$$
 (1)

Because each spring experiences the same force F,

$$u_1 = \frac{F}{k_1}, u_2 = \frac{F}{k_2}.$$
 (2)

Combining the two previous sets of equations, eliminating F and solving for K, the overall stiffness of the system can be written as:

$$K = \frac{k_1 k_2}{k_1 + k_2}.$$
 (3)

If two springs of equal stiffness $k_1 = k_2$ are put in series, the total system stiffness K will be reduced by a factor of 2. If $k_1 = 100^*k_2$, then k_1 will have little effect on the overall system stiffness K. Additional elements in series provide additional contributions in Equation (3), causing a reduction in the total system stiffness regardless of their individual stiffness. This is important to keep in mind when testing graft materials, because tensile test data provide a measurement of the overall system stiffness. If the spring elements represent real components of a bone-graft system such as tendon, suture, or the fixation device-bone interface, their tensile behavior will be nonlinear in general, that is, k_1 and k_2 will vary with elongation.

TESTING METHODS

Several types of tests are available to assess the relative performance of ligament fixation devices. Failure testing is designed to simulate the response of the fixation device to a sudden overload event. The relevant data obtained from these tests are the initial failure load, the initial failure elongation, the stiffness, and the failure mode. Cyclic testing provides an indication of how repetitive loading will affect the fixation. In vitro kinematic testing assesses the ability of the graft-fixation complex to restore normal joint kinematics (joint signature).

Cross-sectional area measurement

The cross-sectional area of a graft used for tests of ligament fixation devices affects the ultimate load and stiffness properties of the complex. More material of a similar quality in cross-section yields a higher stiffness for the complex and a higher ultimate load if failure occurs within the graft. It is good practice to document the cross-sectional area of the grafts to ensure that it does not bias the results of comparisons between fixation devices, and the measurement is necessary if material property measurement is desired for the graft. Many different approaches have been used. In the 1960s, many investigators used the gravimetric method, which calculated the cross-sectional area through division of the volume by the length.^{1,20} Conventional length measurement methods, such as Vernier calipers, also have been used to measure the width and thickness of ligaments and tendons, and the area has been calculated assuming a rectangular or elliptical cross-sectional shape.13

Area micrometer systems have been popular for area measurement. The system compresses the

specimen into a rectangular slot of known width and measures the specimen height with a micrometer.^{4,10} The area measurements depend on the amount of pressure applied to the system, and thus the technique tends to underestimate the crosssectional area.²

Because of the limitations of caliper and area micrometer measurements, other investigators have used noncontact methods. Ellis⁹ used a "shadow amplitude method" to determine the radius of specimen profiles for cross-section reconstruction. Gupta et al.¹² used a rotating microscope to determine ligament profiles. Lee and Woo¹⁷ described a laser micrometer system that measured tissue profiles in 3° increments as the specimen was rotated through 180°. Although all of these methods provide accurate noncontact measurement of area, digital calipers remain the most commonly used technique for cross-sectional area estimation because calipers are readily available and easy to use.

Displacement and strain measurement

Depending on the particular fixation device and soft tissue graft being tested, there are several options for measurement of elongation. If the goal is to monitor elongation of the overall complex, including the graft, the fixation device, and the attachment to bone, crosshead displacement from the material test machine can be used to determine elongation. One must be careful to ensure that the stiffness of any clamping fixtures is much higher than that of the complex. Typically, the clamp stiffness should be an order of magnitude larger than that of the system being measured. One must also ensure that there is not any "slop" in the clamping system that could result in overestimation of the elongation.

Strain is defined as the elongation of the soft tissue normalized by the initial length. To obtain the strain properties of the ligament or tendon without contributions from the bony insertions or clamp slippage, it is necessary to examine the strain of the ligament or tendon substance. Noncontact methods have become the standard for strain measurement in soft tissues. Woo et al.²¹ pioneered the use of the video dimension analyzer (VDA) for noncontact measurement of ligament strain. That method relied on the placement of contrast markers on the ligament surface that could be tracked using a video device. The device triggered on the transitions from light to dark on particular scan lines within the video image, converting the distance to an output voltage. The VDA could be calibrated to directly output ligament strain for either a live or videotaped tensile test. Many similar techniques have been used since this original study, all relying on the tracking of two or more contrast markers on the ligament surface to determine strain during the test.

Control of the testing environment

The testing environment can have a significant effect on the structural and material properties of ligaments, tendons, and bone. It is important for ligaments, tendons, and bone to remain hydrated during testing. This can be achieved by testing in a saline bath, by keeping the soft tissue covered with saline-soaked gauze, or by using regular sprays of saline mist. A decrease in ligament hydration has been shown to cause a decrease in tissue stiffness and strength.¹⁴ Increased hydration also has been shown to cause a faster drop in load during stress relaxation tests.^{6,15} These effects can be especially pronounced for tests requiring a large amount of time, such as cyclic fatigue testing. Temperature also affects material properties, but not as strongly as hydration. Increases in temperature are accompanied by a decrease in tissue stiffness.²³ The use of a heated saline bath allows simultaneous control of hydration and temperature.

Failure tests

Failure tests are designed to simulate a sudden overload event. A high rate of load or strain application can be obtained using a servohydraulic testing system. These tests provide answers to important questions regarding ligament fixation techniques. The weakest link in the fixation system is easily identified by the location of failure during the test. In addition, by monitoring both applied load and elongation of the complex, the overall system stiffness can be determined as the slope of the linear portion of the load-elongation curve. If optical or other length measurement techniques are applied to individual components of the fixation system, such as the graft or suture, it is possible to estimate the stiffness contribution from the components. Perhaps the most widely quoted (and most misunderstood) result obtained from failure testing is the initial failure load, often confused with ultimate load. Based on the discussions preceding this section, it should be clear that fixation system stiffness is just as important as initial failure load, if not more so. A fixation system that has an initial failure load of 1000 N is really of little use if it



FIG. 3. Schematic representing the load-time and elongation-time curves obtained from a cyclic creep test.

stretches 10 mm before reaching that load. Often, a preconditioning protocol is used before failure testing is performed. This involves 10 or more subfailure loading/unloading cycles to a nominal load level. Preconditioning provides a consistent starting point for comparisons between different fixation techniques. Orientation of the bone or bones with respect to the graft can have a significant effect on the results of failure tests. For more information on the effect of graft orientation in the case of the knee joint, see the article by Woo et al.²²

Cyclic loading

Cyclic fatigue testing evaluates the ability of a fixation system to resist migration under repeated subfailure loading conditions. Creep describes the increases in elongation that are observed when a test complex is subjected to a sustained load or cyclic loading between constant load levels. Stress or load relaxation describes the decrease in stress or load that is observed when a test complex is subjected to elongation at a constant length or cyclic elongation between constant lengths. Cyclic creep testing is considered a demanding test of fixation devices, because the load levels do not change. In the in vivo case, other structures would begin to take up the load as the graft stretched out, relieving the total load applied to the graft. If the fixation slips under cyclic creep testing, it normally continues to slip each cycle until complete failure of the fixation is observed.

Figure 3 presents representative data from a cyclic creep test. (The amount of migration is exaggerated for illustration purposes.) In this case, a triangular load profile is applied to the complex, between limits of 30 N and 150 N. The lower graph illustrates the cyclic migration that would be observed during such a test. Both peak and valley lengths increase with cycle number, as illustrated by the positive slope of the dotted lines in the figure. The peak and valley lengths can be plotted as a function of cycle number for comparison of different fixation devices.

Our laboratory protocol for comparison of ACL fixation devices using reconstructed human or bovine knees involves cyclic creep loading. The loading intensity and duration are based on information regarding rehabilitation protocols at The Orthopedic Specialty Hospital in Salt Lake City, Utah. Patients with recently reconstructed ACLs are subjected to non-weightbearing flexion-extension cycles during the first 3 weeks of physical therapy to assist in the restoration of range of motion and to prevent adhesions. The flexion angle range used during this rehabilitation is 0° to 90°, with each patient performing 900 cycles per week. Thirty degrees was chosen as a reasonable angle at which to perform the cyclic tensile testing, representing an angle at which the in situ strain on the ACL graft is nominal. Specimens are secured in a Plexiglas tank filled with 0.9% normal saline. The tank is attached to the materials test machine. Using a high-magnification lens, a video camera is focused on the contrast markers attached to the soft tissue graft and bone at the entrance of the graft into the tunnel. The video magnification is adjusted to maximize the percentage of the screen occupied by the markers to achieve the best accuracy and resolution for video-based length measurements. A reference length is filmed in the same focal plane as the graft and bone markers to allow determination of physical lengths between the markers using a video motion analysis system. The graft-anchor-bone complex is cyclically loaded between limits of 30 N and 150 N at a rate of 0.5 Hz for a total of 1000 cycles (approximately 30 minutes). Previous work on the cyclic loading of soft tissue ACL reconstructions has shown that peak loads ranging from 50 N to 300 N cause significant graft migration with respect to the graft tunnel lip.¹⁶ Thus, 150 N was chosen as a reasonable level for cyclic testing. The number of cycles was chosen to be consistent with the rehabilitation protocol of 900 cycles per week, approximating a single week of flexion-extension loading on the reconstructed graft. Loading rate

was chosen to allow a single test to be completed in approximately 30 minutes, to minimize soft tissue degradation in the 37°C saline bath. Loadelongation data are collected for the first 100 cycles and then for 10 cycles out of every 100 cycles until the graft-anchor-bone complex fails or 1000 cycles is reached. Video data are collected continuously. Analog load and displacement signals from the test machine are acquired on a personal computer. During all testing, the temperature of the saline tank is maintained at 37°C through a recirculating heater.

A motion analysis software system is used to determine the distance between the bone and soft tissue markers at the peak (150 N) and valley (30 N) of load application at each data collection interval. From these measurements and the initial measurements of distance between the markers at the start of the test, the migration of the graft with respect to the bone (in millimeters) is determined. Data are typically presented to show migration as a function of cycles.

In vitro measurement of joint kinematics

The restoration of normal joint kinematics is one of the goals of ligament reconstruction procedures. Reconstruction of joints in vitro using different fixation techniques can provide the starting point for comparisons of how these devices will perform initially in vivo. Joints such as the knee are six degrees of freedom (DOF) joints, and special care must be taken to allow and constrain appropriate degrees of freedom when measuring joint kinematics in vitro. Testing of, for instance, the anteriorposterior (A-P) laxity of a knee joint with intact ACL and after reconstruction using some type of fixation device can allow the efficacy of the reconstruction procedure and fixation device to be evaluated. In testing A-P knee laxity, it has become customary to constrain the flexion angle, apply the anterior-posterior motion or force, and leave proximal-distal translation, medial-lateral translation, tibial axial rotation, and varus-valgus rotation free. This "5 DOF" test setup is described in detail by Beynnon and Amis,3 including a suggested test protocol. These fixtures are typically designed to interface with a material testing machine, whereby applied A-P loads and displacements can be carefully monitored.

Rate effects

Strain rate has been shown to affect the measured ligament/tendon material properties. Although the stress-strain behavior of many soft tissues is relatively insensitive to strain rate over several decades of variation,¹¹ high rate loading will produce a stiffening response.^{8,24} Cancellous and cortical bone are extremely sensitive to strain rate, with increases in tensile/compressive strength and elastic modulus noted for increases in strain rate.^{7,19} Because of these rate effects, it is important to use similar loading rates for all specimens in a particular study so that results can be directly compared.

COMPLICATIONS OF COMPARISONS WITH HUMAN TISSUE

Although testing with human cadaveric tissue provides the closest anatomy to that encountered in the clinical setting, the material properties of cadaveric cancellous bone, cortical bone, and tendons/ligaments can vary greatly among donors. Donor tissue is obtained from a wide range of individuals, but especially from elderly donors. Cancellous and cortical bone density can be drastically reduced compared with that in the younger population that typically receives ligament grafts. When using cadaveric tissue for evaluations of the initial fixation strength of ligament fixation devices, it is always good practice to document bone density for each specimen and examine any correlations between the tensile test results and density. This is especially important when testing fixation devices that rely on cancellous bone for fixation strength. Beynnon and Amis³ suggest testing male specimens below 65 years of age, and female specimens below 50 years of age to minimize this problem.

Many investigators are beginning to use porcine or bovine hard tissue for evaluation of fixation devices in the case of ACL reconstruction. This eliminates the potential variability introduced as a result of large differences in bone density between donors. The bovine knee joint is much larger than the human knee, and graft tissue such as semitendinosus or gracilis tendons must be harvested from human donors. Although the results cannot be directly compared with studies using human tissue, we have found the use of bovine tissue to be helpful in comparisons between different fixation devices.

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

Statistical analysis is necessary to prove or disprove hypotheses, and to generate new hypotheses. The design of a good experiment depends on proper choice of statistical procedures and the levels of statistical significance and power. The interpretation and control of statistical error requires an understanding of the null hypothesis and the two types of statistical error that can occur. Typically, a hypothesis is put forward that must be tested by the statistic, such as "fixation device A yields a higher ultimate load than fixation device B when used for femoral ACL graft fixation." The corresponding null hypothesis is "there is no difference between the ultimate load for fixation devices A and B."

Type I error describes the case when the null hypothesis is mistakenly rejected. For the example just cited, this corresponds to concluding that there is a significant difference between the ultimate loads achieved using fixation devices A and B, when in fact there is not. The likelihood of making this type of error is controlled by the significance level, often referred to as the p value. The p value gives the probability of committing Type I error. If a p value of 0.05 is chosen, this means the investigator is willing to commit Type I error 5% of the time. There is nothing special about this value—in some cases a larger or smaller value may be appropriate.

Type II error describes the case when the null hypothesis is mistakenly accepted. For the example above, this corresponds to concluding that there is no significant difference between fixation devices A and B, when in fact there is a difference. Type II error is controlled by the statistical power, sometimes represented as β . The statistical power is proportional to σ^2 , the square of the standard deviation, $1/\delta^2$, the squared inverse of the desired detectable difference, and n, the sample size. Because the sample size often is quite low and the standard deviation quite high in experimental studies involving mechanical testing of cadaveric or animal tissue, it is not uncommon for the statistical power to be low for these studies. Values of 0.20 or lower are sometimes seen. For the fixation example, this would indicate that although no difference between devices A and B was detected, the confidence level for this conclusion was only 20%. The most obvious way to improve the statistical power is to increase the sample size. Alternatively, a reduction in systematic errors in measurement will reduce the measured standard deviation. A detailed discussion of p value and statistical power can be found in Lieber.18

CONCLUSION

This article has provided an overview of mechanical testing techniques that can be used for the comparison and evaluation of ligament fixation devices. Each test offers unique information about the fixation device. Failure testing evaluates the initial strength and stiffness of the overall system. Cyclic testing provides information regarding potential slippage of the fixation during rehabilitation or activities of daily living in the early postoperative period before graft incorporation has been achieved. Kinematic testing provides an opportunity to examine the ability of the fixation and reconstruction to restore normal joint kinematics and joint signature. The use of all three test procedures provides a well-rounded picture of how a ligament fixation device will perform before graft incorporation has taken place.

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