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Review

Biomechanics of knee ligaments: injury, healing, and repair

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Abstract

Knee ligament injuries are common, particularly in sports and sports related activities. Rupture of these ligaments upsets the balance between knee mobility and stability, resulting in abnormal knee kinematics and damage to other tissues in and around the joint that lead to morbidity and pain. During the past three decades, significant advances have been made in characterizing the biomechanical and biochemical properties of knee ligaments as an individual component as well as their contribution to joint function. Further, significant knowledge on the healing process and replacement of ligaments after rupture have helped to evaluate the effectiveness of various treatment procedures.

This review paper provides an overview of the current biological and biomechanical knowledge on normal knee ligaments, as well as ligament healing and reconstruction following injury. Further, it deals with new and exciting functional tissue engineering approaches (ex. growth factors, gene transfer and gene therapy, cell therapy, mechanical factors, and the use of scaffolding materials) aimed at improving the healing of ligaments as well as the interface between a replacement graft and bone. In addition, it explores the anatomical, biological and functional perspectives of current reconstruction procedures. Through the utilization of robotics technology and computational modeling, there is a better understanding of the kinematics of the knee and the in situ forces in knee ligaments and replacement grafts.

The research summarized here is multidisciplinary and cutting edge that will ultimately help improve the treatment of ligament injuries. The material presented should serve as an inspiration to future investigators.

Keywords: Biomechanics; Knee ligaments; Tissue engineering; Healing

Contents

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1. Introduction

Injuries to knee ligaments are very common. It has been estimated that the incidence could be at 2/1000 people per year in the general population (Miyasaka et al., 1991) and a much higher rate for those involved in sports activities (Bruesch and Holzach, 1993). Ninety percent of knee ligament injuries involve the anterior cruciate ligament (ACL) and the medial collateral ligament (MCL) (Miyasaka et al., 1991). In fact, recent studies have documented that ACL injuries in females are reaching epidemic proportions with the frequency of rupture more than 3 times greater than that of their male counterparts (Anderson et al., 2001; Arendt and Dick, 1995; Powell and Barber-Foss, 2000). The results of ligament injuries can be devastating. Frequently, surgery is required, but the outcomes are variable. Further, post-surgical rehabilitation could require an extended absence from work or athletic competition.

Basic science and clinical studies have revealed that a ruptured MCL can heal spontaneously (Frank et al., 1983; Indelicato, 1983; Jokl et al., 1984; Kannus, 1988). However, laboratory studies have shown that its ultrastructure and biochemical composition remain significantly altered (Frank et al., 1983; Niyibizi et al., 2000; Weiss et al., 1991). Furthermore, the mechanical properties of the ligament substance remain substantially inferior to those of normal ligaments even after years of remodeling (Loitz-Ramage et al., 1997; Ohland et al., 1991). On the other hand, midsubstance tears of the ACL and posterior cruciate ligament (PCL) would not heal spontaneously and surgical reconstruction using a replacement graft is often required (Hirshman et al., 1990; Kannus and Jarvinen, 1987). While the majority of ligament reconstructions yield good short-term clinical results, 20–25% of patients experience complications including instability that could progressively damage other knee structures (Aglietti et al., 1997; Bach et al., 1998; Daniel et al., 1994; Jomha et al., 1999; Ritchie and Parker, 1996; Shelbourne et al., 1995; Yagi et al., 2002).

Thus, there has been a tremendous quest for knowledge to better understand ligament injuries, healing and remodeling in hope to develop new and improved treatment strategies. The needs in meeting this goal have stimulated researchers to seek new and innovative methods of investigation. Because of the complex biological process, it has become clear that collaborations from different disciplines rather than an individualistic approach in research must be developed. In this review, the properties of normal ligaments, including their anatomical, biological, biochemical and mechanical properties, as well as the changes that occur following injury will be described. The MCL will be used as a model because of its uniform cross-sectional area, large aspect ratio, and propensity for healing. Subsequently, novel functional tissue engineering methodologies and some of the early findings will be presented. The challenging problems which remain to be solved and the potential of new treatment strategies will be explored. In terms of ligament reconstruction, the biomechanics of surgical reconstruction of the ACL and the utilization of robotics technology to study some of the key surgical parameters that affect the performance of the replacement grafts will be reviewed. It is hoped that these creative research approaches will inspire many to join this course of investigation and ultimately help improve the treatment of ligament injuries.

2. Anatomy, histological appearance and biochemical constituents of normal ligaments

Ligaments are composed of closely packed collagen fiber bundles oriented in a parallel fashion to provide for stability of joints in the musculoskeletal system. The major cell type is the fibroblast and they are interspersed in the parallel bundles of collagen.

In the human knee, the MCL is approximately 80 mm long and runs from the medial femoral epicondyle distally and anteriorly to the posteromedial margin of the metaphysis of the tibia. The lateral collateral ligament (LCL) originates from the lateral femoral epicondyle and passes postero-distally to the top of the fibular head. The cruciate ligaments, which are named anterior and posterior according to their site of attachment to the tibia, are located within the capsule and cross each other obliquely. The anterior cruciate ligament (ACL) arises from the anterior part of the intercondylar eminentia of the tibia and extends to the posterolateral aspect of the intercondylar fossa of the femur. The posterior cruciate ligament (PCL) arises from the posterior part of the intercondylar eminentia of the tibia and passes to the anterolateral aspect of the intercondylar fossa of the femur. Although morphologically intraarticular, the cruciate ligaments are surrounded by a synovial layer. The ACL consists of two bundles, an anteromedial (AM) and a posterolateral (PL) bundle. The AM bundle is thought to be important as a restraint to anterior–posterior translation of the knee, while the PL bundle is thought to be an important restraint to rotational moments about the knee (Yagi et al., 2002). This anatomic division of these bundles is based on the gross tensioning pattern of the ACL during passive flexion-extension of the knee, with the AM bundle being tauter in flexion and the PL bundle tauter in extension. The PCL is also composed of two distinct bundles, the antero-lateral (AL) and the postero-medial (PM) bundle. Additionally, ligaments are sometimes found anterior and posterior to the PCL in some people. They are the anterior meniscofemoral ligament (MFL; i.e. ligamentum Humphrey) and the posterior meniscofemoral ligament (i.e. ligamentum Wrisberg) (Girgis et al., 1975).

Generally, ligaments are inserted to bone in two ways; direct and indirect (Fig. 1). For direct insertions (e.g. the femoral insertion of MCL), fibers attach directly into the bone and the transition of ligament to bone occurs in four zones: ligament, fibrocartilage, mineralized fibrocartilage and bone (Woo et al., 1987). For an indirect insertion (e.g. the tibial insertion of MCL) superficial fibers are attached to periosteum while the deeper fibers are directly attached to the bone at acute angles (Woo et al., 1987). The tibial insertion of the MCL crosses the epiphyseal plate so that it can be lengthened in synchrony with the bone growth.

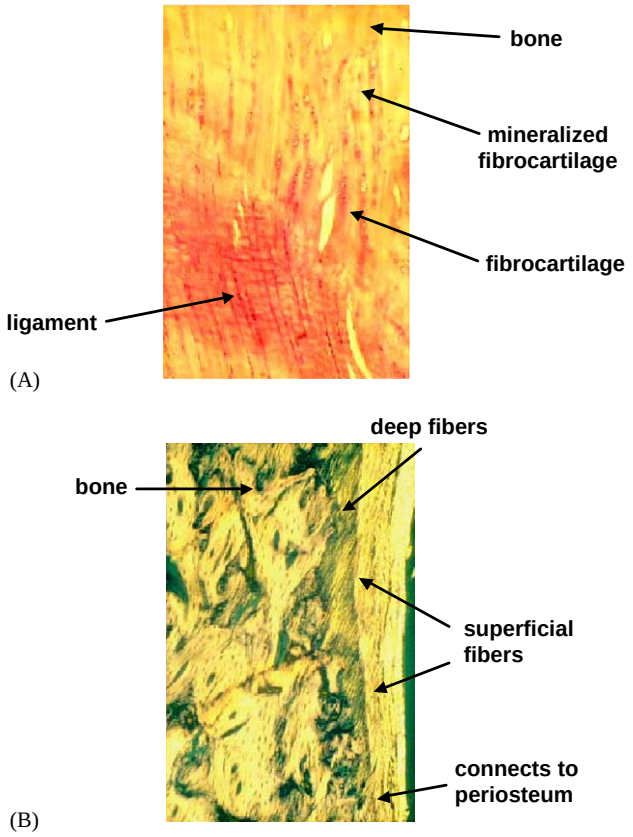

Fig. 1. (A) Photomicrograph demonstrating direct insertion, i.e. the femoral insertion of rabbit medial collateral ligament (MCL). (B) Photomicrograph demonstrating indirect insertion, i.e. the tibial insertion of rabbit MCL. (Hematoxylin and eosin, x50) (permission requested from (Woo et al., 1987)).

Between 65 and 70% of a ligament's total weight is composed of water. On a fat-free basis, Type I collagen is the major constituent (70–80% dry weight) and is primarily responsible for a ligament's tensile strength. Type III collagen (8% dry weight) and Type V collagen (12% dry weight) are other major components (Birk and Mayne, 1997; Linsenmayer et al., 1993). Collagen Types II, IX, X, XI, and XII have also been found to be present (Fukuta et al., 1998; Niyibizi et al., 1996; Sagarriga Visconti et al., 1996).

Variations in the concentrations of these basic constituents lead to a diverse array of mechanical behaviors of knee ligaments that are suitable for their respective functions. A comparative study showed that the tangent modulus and tensile strength of the rabbit MCL is higher than the ACL (Woo et al., 1992) which correlates with a larger mean fibril diameter for the MCL (Hart et al., 1992). In addition, the fibroblasts of the MCL are more spindle shaped (Lyon et al., 1991) and produce a higher level of procollagen type I mRNA (Wiig et al., 1991) and a lower collagen type III to type I ratio in culture (Ross et al., 1990). Further, mechanical loading has been found to regulate the gene expression

of collagens in ligaments (Hsieh et al., 2002). Therefore, each ligament's composition is directly correlated with its mechanical properties.

3. Tensile properties of ligaments

Ligaments are best suited to transfer load from bone to bone along the longitudinal direction of the ligament. Thus, their properties are commonly studied via a uniaxial tensile test of a bone–ligament–bone complex (e.g. femur-MCL-tibia complex). These tests result in a load–elongation curve that is non-linear and concave upward. This enables ligaments to help to maintain smooth movement of joints under normal, physiologic circumstances and to restrain excessive joint displacements under high loads. The parameters describing the structural properties of the bone–ligament–bone complex include stiffness, ultimate load, ultimate elongation, and energy absorbed at failure. With cross-sectional area and strain measurements, a stress–strain curve representing the mechanical properties (quality) of the ligamentous tissue can be obtained. The parameters describing the mechanical properties of the ligament substance include tangent modulus, ultimate tensile strength, ultimate strain, and strain energy density. A large number of experimental methods have been employed by investigators to overcome some of the technical difficulties encountered in measuring the mechanical properties of ligaments (Beynnon et al., 1992; Ellis, 1969; Lam et al., 1992; Lee and Woo, 1988; Peterson et al., 1987; Peterson and Woo, 1986; Smutz et al., 1996). Furthermore, environmental factors can also cause large differences in the experimental data obtained (Crowninshield and Pope, 1976; Figgie et al., 1986; Haut, 1983; Haut and Powlison, 1990; Noyes et al., 1974). For more information on these methodologies and environmental factors, the readers are encouraged to read the provided references and study the chapter entitled: Biology, Healing and Repair of Ligaments in Biology and Biomechanics of the Traumatized Synovial Joint: The Knee as a Model, 1992 by the authors (Woo et al., 1992).

An equally important consideration is the geometry of the ligament. Unlike the MCL whose cross-section is relatively uniform over its length, the ACL and PCL have two functionally distinct bundles that are loaded non-uniformly (Fuss, 1989; Girgis et al., 1975; Sakane et al., 1997). Thus, they need to be separated in order to have a specimen with a more uniform cross-sectional area for tensile testing. Using this approach, a study performed at our center showed the tangent modulus of a section of the rabbit ACL (516 ± 64 MPa) was less than half of that for the rabbit MCL (1120 ± 153 MPa) (Woo et al., 1992). Further, the tangent modulus, tensile strength, and strain energy density of the AM bundle in the human ACL was larger than that for the PL bundle (Butler et al., 1992). In a separate study, the mechanical properties of the bundles of the human PCL were found to be different as well (Harner et al., 1995). The tangent modulus of the AL bundle (294 ± 115 MPa) was almost twice that of the PM bundle (150 ± 69 MPa). The fact that different bundles have different properties suggests that each bundle contributes to knee joint stability differently, which may have important ramifications on their replacements (Table 1).

3.1. Ligament anisotropy

Ligaments are three dimensional (3-D) anisotropic structures. To describe the 3-D mechanical behavior of the human MCL, investigators have developed a quasi-static hyperelastic strain energy model based on the assumption of transverse isotropy (Quapp and Weiss, 1998). The total strain energy, W, in response to a stretch along the collagen fiber direction, λ, was defined to be equal to the sum of the strain energy resulting from ground substance (F_1), collagen fibers (F_2), and an interaction component (F_3),

$$W(I_1, I_2, \lambda) = F_1(I_1, I_2) + F_2(\lambda) + F_3(I_1, I_2, \lambda) \quad (1)$$

where I_1 and I_2 are invariants of the right Cauchy stretch tensor. For a uniaxial tensile test, F_1 was described with a two coefficient Mooney–Rivlin material model

$$F_1 = 1/2[C_1(I_1 - 3) + C_2(I_2 - 3)], \quad (2)$$

where C_1 and C_2 are constants, and F_2 was described by separate exponential and linear functions. F_3 was assumed to be zero.

The Cauchy stress, $\mathbf{T}$, can then be written as

$$\mathbf{T} = 2\{(W_1 + I_1 W_2)\mathbf{B} - W_2\mathbf{B}^2\} + \lambda W_\lambda \mathbf{a} \otimes \mathbf{a} + \rho\mathbf{1}, \quad (3)$$

where, $\mathbf{B}$ is the left deformation tensor, and W_1, W_2, and W_λ are the partial derivatives of strain energy with respect to I_1, I_2, and λ, respectively. The unit vector field, $\mathbf{a}$, represents the fiber direction in the deformed state, and ρ is the hydrostatic pressure required to enforce incompressibility.

It was found that this constitutive model can fit both the data obtained from longitudinal and transverse dumbbell shaped specimens cut from the human MCL

Table 1
Values for tangent modulus of the human MCL (Quapp and Weiss, 1998), AM and PL bundles of the human ACL (Butler et al., 1992), and AL and PM bundles of the PCL (Harner et al., 1995).

Tangent modulus (MPa)				
Human MCL	Human ACL		Human PCL	
	AM	PL	AL	PM
332 ± 58	283 ± 114	154 ± 120	294 ± 115	150 ± 69

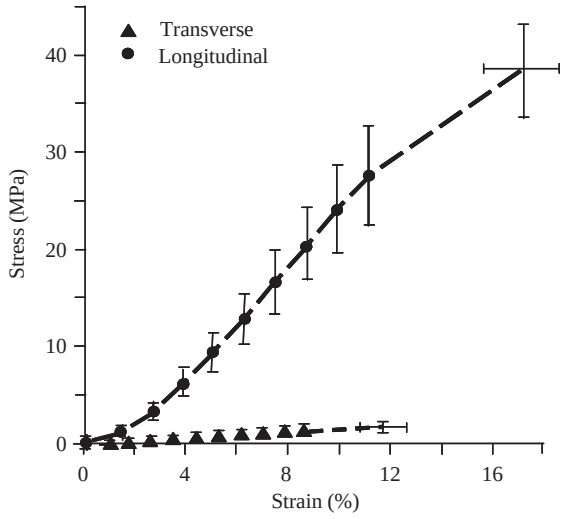

Fig. 2. Stress–strain curves for human MCLs longitudinal and transverse to the collagen fiber direction (permission requested from Quapp and Weiss (1998)).

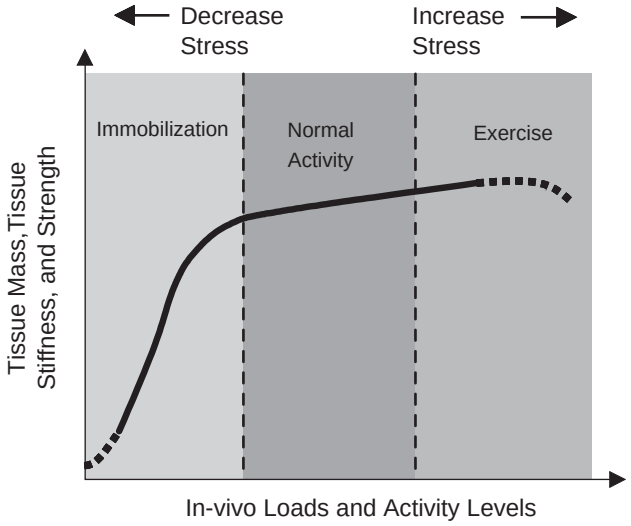

Fig. 3. A schematic diagram describing the homeostatic responses of ligaments and tendons in response to different levels of stress and motion (permission requested from (Woo et al., 1987)).

(Fig. 2). The longitudinal specimens displayed a tangent modulus of 332.2 ± 58.3 MPa and a tensile strength of 38.6 ± 4.8 MPa, while the transverse specimens were an order of magnitude lower with a tangent modulus of 11.0 ± 0.9 MPa and tensile strength of 1.7 ± 0.5 MPa (Quapp and Weiss, 1998).

3.2. Significant biological factors on the properties of ligaments

The effects of immobilization and exercise on the mechanical properties of ligaments has been investigated by a number of laboratories (Larsen et al., 1987; Newton et al., 1990; Noyes, 1977; Woo et al., 1987). When rabbit hind limbs were subjected to a few weeks of immobilization, there were marked decreases in the structural properties of the femur–MCL–tibia complex (FMTC). These decreases occurred due to subperiosteal bone resorption within the insertion sites, as well as microstructural changes in the ligament substance. Remobilization was found to reverse these negative changes. However, up to one year of remobilization was required for the properties of the ligament to return to normal levels following 9 weeks of immobilization (Woo et al., 1987). Similar results were found for the femur–ACL–tibia complex (FATC) of primates and rabbits (Newton et al., 1990; Noyes, 1977). Long periods of exercise training, on the other hand, only showed marginal increases in the structural properties of ligaments with a 14% increase in linear stiffness of the FMTC and a 38% increase in ultimate load/body weight (Laros et al., 1971; Woo et al., 1982, 1979). There was only a slight change in the mechanical properties of the ligament substance.

Based on the results of these and other related studies, a highly non-linear representation of the relationship between different levels of stress and ligament properties is depicted in Fig. 3. The normal range of physiological activities is represented by the middle of the curve. Immobilization results in a rapid reduction in tissue properties and mass. In contrast, long term exercise resulted in a slight increase in mechanical properties as compared with those observed in normal physiological activities.

Skeletal maturity also causes significant changes to ligaments whereby the stiffness and ultimate load of the FMTC was shown to increased dramatically from 6 to 12 months of age followed by insignificant change from 1 to 4 years in the rabbit model (Woo et al., 1990). This corresponded with a change in failure mode from the tibial insertion to the midsubstance reflecting closure of the tibial epiphysis during maturation (Woo et al., 1986). On the other hand, the human FATC demonstrated a significant decrease in the stiffness and ultimate load with increasing age (Noyes and Grood, 1976; Woo et al., 1991). Therefore, each ligament is unique in its growth, development, and aging. Investigators should be cautious when extrapolating age related changes from one ligament (ex. ACL to PCL) or species (ex. rabbit to human) to another.

4. Viscoelastic properties of ligaments

The complex interactions of collagen with elastin, proteoglycans, ground substance, and water results in the time- and history-dependent viscoelastic behaviors of ligaments. In response to various tensile loading

protocols, ligaments exhibit hysteresis (i.e. internal energy dissipation), creep, and stress relaxation. The following is a comprehensive review of the theories to describe these properties.

4.1. The quasi-linear viscoelastic theory

The quasi-linear viscoelastic (QLV) theory developed by Fung (Fung, 1993) is one of the most successful models to describe the time- and history-dependent viscoelastic properties of soft tissues (Carew et al., 1999; Kim et al., 1999; Simon et al., 1984; Zheng and Mak, 1999), especially ligaments (Abramowitch and Woo, 2004; Funk et al., 2000; Kwan et al., 1993; Woo et al., 1981) and tendons (Elliott et al., 2003; Thomopoulos et al., 2003). The theory assumes that a non-linear elastic response and a separate time-dependent relaxation function can be combined in a convolution integral to result in a 1-D general viscoelastic model expressed as follows:

$$\sigma(t) = \int_{-\infty}^{t} G(t-\tau)\,\frac{\partial \sigma^{e}(\varepsilon)}{\partial \varepsilon}\,\frac{\partial \varepsilon}{\partial \tau}\,\partial\tau. \tag{4}$$

The elastic response is a strain dependant function. One of the representations can be written as follows:

$$\sigma^{e}(\varepsilon) = A(e^{B\varepsilon} - 1). \tag{5}$$

Using Fung's generalized relaxation function based on the assumption of a continuous relaxation spectrum, the time-dependent reduced relaxation function, $G(t)$ (Fung, 1993), takes the form

$$G(t) = \frac{[1 + C\{E_1(t/\tau_2) - E_1(t/\tau_1)\}]}{[1 + C^{*}\mathrm{Ln}(\tau_2/\tau_1)]}, \tag{6}$$

where E_1 is the exponential integral, $\int_y^{\infty} e^{-z}/z\,\mathrm{d}z$, and, C, τ_1 and τ_2 are constants with $\tau_1 \ll \tau_2$.

Using this approach, the QLV theory has been utilized to model the canine MCL (Woo et al., 1981). Based on separate curve fitting of $\sigma^{e}(\varepsilon)$ and $G(t)$ to the loading and relaxation portions of the experimental data, respectively, the constants of the QLV theory were obtained. These constants were then employed to successfully predict the peak and valley stress values of a cyclic stress relaxation experiment of canine FMTCs.

It should be noted, however, that the theory has been developed based on the assumption of a idealized step-change in strain which is impossible to apply experimentally. Therefore, there are significant errors that could occur in determining the viscoelastic constants, especially τ_1 (Dortmans et al., 1984; Funk et al., 2000). Previous methods to account for these errors include, normalization procedures, iterative techniques, extrapolation and deconvolution, as well as directly fitting the measured strain history (Carew et al., 1999; Doehring et al., 2004; Funk et al., 2000; Kwan et al., 1993; Myers et al., 1991; Nigul and Nigul, 1987). Recently, our research center has developed an alternative approach whereby the QLV theory can be applied to experiments which utilize a slow-strain rate in order to avoid experimental errors such as overshoot and vibrations (Abramowitch and Woo, 2004). Using Boltzmann's superposition principle, it can be shown that the loading portion of a stress relaxation experiment with a linear strain history and strain rate, γ, for $0 < t < t_0$ can be described by:

$$\sigma(t) = \frac{AB\gamma}{1 + C\,\ln(\tau_2/\tau_1)} \times \int_0^{t} \{1 + C(E_1[(t-\tau)/\tau_2]E_1[(t-\tau)/\tau_1])\} \times e^{B\gamma\tau}\partial\tau. \tag{7}$$

Similarly, the subsequent stress relaxation at a constant strain, from t_0 to $t = \infty$, can be described by changing the upper limit of integration in Eq. (7) from t to t_0,

$$\sigma(t) = \frac{AB\gamma}{1 + C\,\ln(\tau_2/\tau_1)} \times \int_0^{t_0} \{1 + C(E_1[(t-\tau)/\tau_2]E_1[(t-\tau)/\tau_1])\} \times e^{B\gamma\tau}\partial\tau, \tag{8}$$

where A, B, C, τ_1, and τ_2 are material constants to be determined. Simultaneously curve-fitting these equations to the loading and relaxation portions of the data from a stress relaxation experiment and assuming ligaments are relatively insensitive to strain rate allows the constants A, B, C, τ_1, and τ_2 to be determined (Abramowitch and Woo, 2004). Because this approach accounts for relaxation manifested during loading, the errors in the obtained constants resulting from the assumption of an idealized step-elongation are minimized.

Recently, this approach was utilized to describe the viscoelastic behavior of the goat FMTC (Fig. 4). It was found that the obtained constants were improved compared to an approach that assumed an idealized step-elongation. Specifically, constant τ_1 was found to be an order of magnitude lower using the new approach which agrees with the results of a previous study that analytically determined errors resulting from assuming an idealized step-elongation (Dortmans et al., 1984). In addition, the obtained constants were verified by the prediction of a second independent experiment whereby a more general cyclic strain history was utilized (Abramowitch and Woo, 2004).

4.2. Continuum based viscoelastic models

The QLV theory assumes that the rate of relaxation remains relatively constant. Recent studies on ligaments from the rat and rabbit have shown that ligament

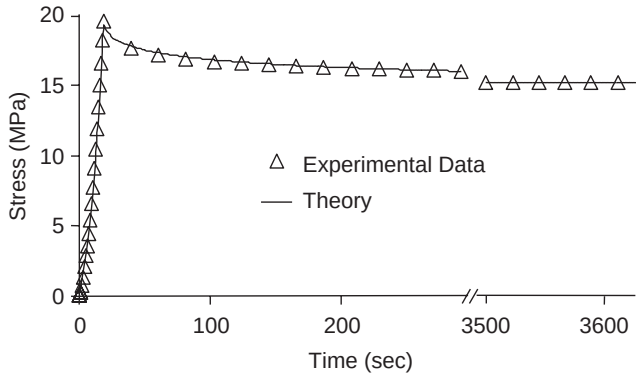

Fig. 4. A typical curve fit using the new approach to experimental data obtained from a stress relaxation test of a goat FMTC (permission requested from Abramowitch and Woo (2004)).

viscoelastic behavior is nonlinear (i.e. the rate of relaxation decreases as the level of applied strain increases up to 2.5% strain) (Hingorani et al., 2004; Provenzano et al., 2001). In addition previous work has demonstrated that the creep and stress relaxation behaviors of the MCL likely arise from different mechanisms (Thornton et al., 1997). In fact, Professor Fung in his book *Biomechanics* (2nd ed; 1993) described this phenomenon by suggesting "…creep is fundamentally more nonlinear, and perhaps does not obey the quasi-linear hypothesis." Thus, alternative viscoelastic models, such as the single integral finite strain (SIFS) theory, have been used to fully describe the 3-D behavior of ligaments (Johnson et al., 1996). The theory is based on the general integral series representation for a nonlinear viscoelastic response (Pipkin and Rogers, 1968). The concepts of microstructural change resulting from recruitment and fading memory to ensure that more recent states of strain have greater weight in determining the stress than earlier states are incorporated. The specific constitutive equation is written as:

$$\begin{aligned}\mathbf{T} = & -p\mathbf{I} + C_0\{[1+\mu I(t)]\mathbf{B}(t) - \mu\mathbf{B}^2(t)\} \\ & -(C_0 - C_\infty)\int_0^t G(t-s) \\ & \times\{[1+\mu I(s)]\mathbf{B}(t) - \mu\mathbf{F}(t)\mathbf{C}(s)\mathbf{F}^{\mathrm{T}}(t)\}\,\mathrm{d}s \end{aligned} \quad (9)$$

where $\mathbf{T}$ is the Cauchy stress, p is the hydrostatic pressure to enforce incompressibility, $\mathbf{I}$ is the identity tensor, $\mathbf{B}$ is the left Cauchy–Green strain tensor, $G(t)$ is the time-dependant relaxation function, C_0 is the instantaneous modulus, and $I(s) = \mathrm{tr}\ \mathbf{C}$, where $\mathbf{C}$ is the right Cauchy–Green strain tensor. The SIFS model can also be linearized to yield the equations for classical linear viscoelasticity and reduces to an appropriate finite elasticity model for time zero.

The model was applied to data from uniaxial extension of younger and older human PTs and canine MCLs (Johnson et al., 1996). Constants were determined from curve-fitting stress–strain and stress–relaxation data and used to predict the time-dependent stress resulting from cyclic loading with good agreement. Thus, SIFS theory can be used to model viscoelastic behavior resulting from large deformations in 3-D. The robustness of this theory makes it useful for many future applications.

5. Healing of knee ligaments

5.1. MCL healing

Because the injured MCL of the knee can heal spontaneously, it has been used as an excellent experiment model for many studies, especially those from the rabbit (Weiss et al., 1991; Woo et al., 1987). These studies have helped to understand that the rate, quality and composition of the healing MCL are dependent on the treatment modality. Conservative treatment of an isolated MCL injury produced better results to those with surgical repair either with or without immobilization (Boorman et al., 1998; Weiss et al., 1991; Woo et al., 1987). Immobilization after ligament injury was shown to lead to a greater percentage of disorganized collagen fibrils, decreased structural properties of the FMTC, decreased mechanical properties of the ligament substance, and slower recovery of the resorbed insertion sites (Woo et al., 1987). Clinical studies have also reported that patients with a complete tear of the MCL respond well to conservative treatment without immobilization by plastercasts (Fetto and Marshall, 1978). As a result, the paradigm of clinical management has shifted from surgical repair with immobilization to nonoperative management with early controlled motion (Indelicato, 1995; Reider et al., 1994).

5.2. Phases of ligament healing

The continuous process of healing following a tear of the MCL can be roughly divided into three overlapping phases (Frank et al., 1983; Oakes, 1982; Weiss et al., 1991). The inflammatory phase is marked by hematoma formation which starts immediately after injury and lasts for a few weeks. It is followed by the reparative phase where fibroblasts proliferate and produce a matrix of proteoglycan and collagen, especially type III collagen, to bridge between the torn ends. Over the next 6 weeks, increasingly organized matrix, predominantly type I collagen, and cellular proliferation occur. Finally, the remodeling phase which is marked by alignment of collagen fibers and increased collagen matrix maturation can continue for years (Frank et al., 1983).

Thus, the constituents of the healing ligament are abnormal even after one year (Weiss et al., 1991). It contains increased amount of proteoglycans, a higher ratio of type V to type I collagen, a decrease in the number of mature collagen crosslinks, and fibrils with homogenously small diameters (~70 nm) (Niyibizi et al., 2000; Plaas et al., 2000; Shrive et al., 1995). Frequently, there is an increase in the number of collagen fibrils of the healed ligament, but the diameters of these fibrils are smaller than those of a normal ligament (Frank et al., 1997).

These changes are reflected in the structural properties of the healing FMTC which are inferior to controls at 12 weeks after injury (Weiss et al., 1991). However, by 52 weeks post-injury the stiffness of the injured FMTC recovered, but the varus–valgus (V–V) rotation of the knee remained elevated and the ultimate load of the FMTC remained lower than those for the sham-operated MCL (Inoue et al., 1990; Loitz-Ramage et al., 1997; Ohland et al., 1991). Concomitantly, the cross-sectional area of the healing ligament measured as much as $2\frac{1}{2}$ times its normal size by 52 weeks (Ohno et al., 1995). Thus, the recovery of the stiffness of the FMTC is largely the result of an increase in tissue quantity.

The mechanical properties of the healing MCL midsubstance remain consistently inferior to those of the normal ligament and do not change with time up to one year (Ohno et al., 1995; Weiss et al., 1991) (Fig. 5). In terms of the viscoelastic properties of the healing MCL, there is increased viscous behavior, reflected by a greater amount of stress relaxation or creep, for the first 3 months after injury. However, some studies suggested that these values returned to normal levels after this time period (Chimich et al., 1991; Woo et al., 1987), while others suggested they remained increased (Newton et al., 1990).

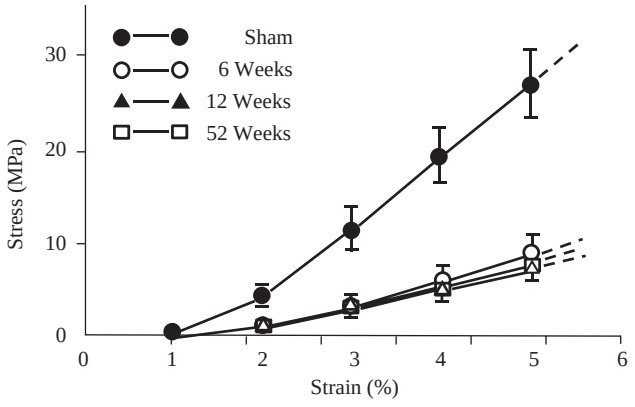

Fig. 5. Stress–Strain curves representing the mechanical properties of the medial collateral ligament substance for sham-operated and healing MCLs at time periods of 6 ($n = 6$), 12 ($n = 6$), and 52 ($n = 4$) weeks (permission requested from (Ohland et al., 1991)).

5.3. New animal model

Animals that are large in size and more robust in activity level, such as the goat model, have also been studied (Ng et al., 1995). The tensile properties of the healing goat FMTC can achieve stiffness and ultimate load that are closer to control values at earlier time periods than the healing rabbit FMTC (Abramowitch et al., 2003a). Yet, the tangent modulus and morphology of the healing ligament for the goat and rabbit models were not different, suggesting that both heal with a similar quality of tissue.

In addition, viscoelastic experiments show that the percentage of stress relaxation of the healing MCL remained twice that of contralateral controls (Abramowitch et al., 2004). Using the QLV theory, it was found that, the initial slope of the elastic response, constants $A * B$, was nearly an order of magnitude lower for the healing MCL. In addition, the healing MCL dissipated more energy, had a longer recovery time upon removal of load, and its long-term relaxation plateaued earlier as dimensionless constant C was nearly 3 times greater for healing MCLs and constant τ_2 was approximately 63% of that for sham-operated controls.

Models to represent injuries to more than one ligament, e.g. MCL & ACL, have also been studied. Using the rabbit model, the healing MCL can benefit from ACL reconstruction, but no long-term advantages were found with primary repair of the MCL (Yamaji et al., 1996). Thus, laboratory data have helped many clinicians to choose to reconstruct the ACL and treat the ruptured MCL non-operatively. Regardless, the structural properties of the FMTC, mechanical properties of the healing MCL, and knee function all remained poorer than those for isolated MCL injuries (Abramowitch et al., 2003c). Clinical data also support these findings (Yamaji et al., 1996).

6. New approaches to improve healing of ligaments—functional tissue engineering

In order to improve the quality of healing tissues and restore the normal function of ligaments, functional tissue engineering based on novel biological and bioengineering techniques has been explored. Examples include the usage of a variety of growth factors, gene transfer and gene therapy, cell therapy, as well as the use of scaffolding materials. Together with mechanical factors, these technologies offer great potential for the utilization of functional tissue engineering in ligament healing.

6.1. Growth factors

By binding to their specific receptors on cell surfaces, growth factors can arouse targeted biological responses.

Studies have shown how the expressions of insulin-like growth factor-I (IGF-I), transforming growth factor (TGF-β), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) are altered in healing ligaments and tendons (Duffy et al., 1995; Panossian et al., 1997; Pierce et al., 1989; Schmidt et al., 1995; Sciore et al., 1998; Steenfos, 1994).

In the early stages of MCL healing, three mammalian isoforms of TGF-β1, β2 and β3, are involved in the healing process. TGF-β1 is increased in and around the wound site seven days following injury (Lee et al., 1998). In vitro studies at our research center demonstrated that the application of TGF-β1 increases collagen synthesis 1.5 fold over controls in both MCL and ACL fibroblasts (Marui et al., 1997). TGF-β2 has been shown to increase the expression of type I collagen at 6 weeks after injury, resulting in a profound increase in healing mass, but with limited increase in the structural properties of the FMTC (i.e. the stiffness but not the load at failure of the healing MCL could be increased) (Spindler et al., 2002, 2003).

PDGF could also play a significant role in the early stages of healing as the application of PDGF-BB improved the structural properties of the rabbit FMTC between 2 and 6 weeks (Batten et al., 1996; Lee et al., 1998). Similar results have been demonstrated in a rat study (Batten et al., 1996). Locally applied PDGF may also improve the mechanical properties of the ipsilateral flexor tendon graft after ACL reconstruction (Weiler et al., 2004).

The potential of synergistic effects of two or more growth factors has been explored. A combination of PDGF-BB/TGF-β1 did not enhance the structural properties of the healing FMTC compared to the use of PDGF-BB alone (Woo et al., 1998). In addition, the PDGF/TGF-β2 combination also had no significant effect compared to the use TGF-β2 alone (Spindler et al., 2003). On the other hand, another study has shown that combined local application of TGF-β1 and EGF could improve the structural properties of the bone-patellar tendon-bone autograft for ACL reconstruction in canine (Yasuda et al., 2004). Clearly, the healing process of ligaments is much more complex than the in vitro cell culture environment and more studies are necessary.

6.2. Gene transfer and gene therapy

Gene transfer using carriers including both retroviral and adenoviral vectors as well as liposomes (Nakamura et al., 1998) have been used to induce DNA fragments into healing ligaments to promote or depress the expression of certain genes in hope to improve their quality.

In our studies, an adenoviral vector appeared to be able to express more effectively in ligaments than retroviral vectors. By using LacZ gene as a marker gene, it was shown that the gene expression could last for 6 weeks in ligaments with the use of adenovirus (Hildebrand et al., 1999). In addition, an in situ gene transfer of TGF-β1 using an adenoviral vector increased the cellularity and enhanced the deposition of Type I and III collagen in a ruptured ACL (Pascher et al., 2004).

A promising method is antisense gene therapy using oligonucleotides (ODNs) to reduce undesirable proteins in the healing ligament. This methodology has been shown to successfully reduce decorin in the healing MCL of a rabbit resulting in increased diameters of the collagen fibrils as well as an 85% increase in the tensile strength of the healing MCL (Nakamura et al., 2000). In our research center, antisense gene therapy was used to reduce the higher level of collagen types III and V in the healing MCL. Preliminary in vitro data revealed that the gene expression of these collagens could be lowered by approximately 40% (Jia et al., 2002, 2001; Shimomura et al., 2002). In vivo studies showed that ODNs were taken up by fibroblasts and reduced the expression of the type V collagen protein. This is indeed a promising and exciting approach that warrants additional studies.

6.3. Cell therapy

Cell therapy using mesenchymal progenitor cells (MPCs) or mesenchymal stem cells (MSCs) also has tremendous potential in tissue engineering. These cells can differentiate into a variety of cell types, including fibroblasts (Lazarus et al., 1995). MSCs isolated from the bone marrow, cultured with or without gene transfer, and finally transplanted to host tissues appear to retain their potential to differentiate (Bruder et al., 1997; Goshima et al., 1991; Haynesworth et al., 1992). For the patellar tendon in rabbits, an autologous MSC-collagen graft could improve the quality as well as accelerate the rate of healing (Awad et al., 2003, 1999). In our research center, it was found that MSCs implanted in the injured MCL of the rat differentiated into fibroblasts. In addition, the cells were found to have migrated to the non-injured area of the ligament after 3 days. These results are encouraging because the MSCs have the potential to serve as a vehicle for delivering therapeutic molecules as well as directly enhance the healing of ligaments (Watanabe et al., 2002).

6.4. Biological scaffolds

There are several biological scaffolds such as gels or membranes made from alginate, chitosan, collagen or hyaluronic acid (Drury and Mooney, 2003; Kim et al., 1998). For ligaments, the porcine small intestinal

submucosa (SIS) has been found to enhance their repair (Badylak et al., 1999; Musahl et al., 2004). SIS is mainly composed of collagen (90% of dry weight) and contains a small amount of cytokines and growth factors such as FGF and TGF-β (Badylak et al., 1999). It is a resorbable scaffold that can hold cells and nutrients necessary for healing as well as to provide a collagenous structure to be remodeled (Badylak et al., 1995).

A study from our research center has demonstrated the enhancement of the biomechanical properties and biochemical compositions of healing ligament by using SIS. The effect of a single layer of SIS treatment of a 6 mm gap injury of the rabbit MCL was examined at 12 and 26 weeks post-surgery. The stiffness of the FMTC was found to increase 56% compared to the non-treated control while the ultimate load also nearly doubled at 12 weeks post injury. Furthermore, the tangent modulus of the healing MCL increased by more than 50% at 12 weeks and this effect persisted up to 26 weeks where the SIS-treated group had a 33% higher tangent modulus and a 49% higher stress at failure. The histological appearance of the SIS treated MCL had increased cellularity, greater collagen density, and improved collagen fiber alignment (Musahl et al., 2004). Correlatively, the ratio of collagen type V/I was decreased with a corresponding increase in collagen fibril diameter. All the results indicate that the application of this potential functional tissue engineering technology to enhance the healing of ligaments is promising.

6.5. Mechanical factors

It is also well-known that mechanical environment can induce changes in the cell behavior and collagen architecture. In vitro, fibroblasts that were mechanically stretched in a microgrooved substrate, i.e an environment designed to mimic the intact ligament, have the tendency to align with the direction of stretch as well as produce better organized collagen matrix (Fig. 6) (Huang et al., 1993; Wang et al., 2003). Therefore, functional tissue engineering with the application of proper mechanical environment may lead to positive changes in the mechanical properties of ligaments.

7. ACL reconstruction

It is hoped that the new knowledge gained from studying and treating healing ligaments may one day lead to alternative strategies for treating other ligaments that do not heal (e.g. ACL and PCL of the knee). For now, however, injuries to the ACL and PCL are managed by ligament reconstruction using replacement auto- or allografts. While many patients have benefited from these transplantations, a large percentage (20–25%) of patients for ACL reconstruction and a higher percentage (up to 60%) for PCL reconstruction, unfortunately, have less than satisfactory outcomes (Lipscomb et al., 1993). Efforts are being made to better understand the kinematics of the knee and the in situ forces in the intact ACL and ACL replacement grafts. To do this, the following section will review the anatomical, biological and functional perspectives of the intact ACL in comparison to current ACL reconstruction procedures and grafts.

7.1. Graft function

Previous literature has documented many methods to measure six degree of freedom (DOF) knee motion and

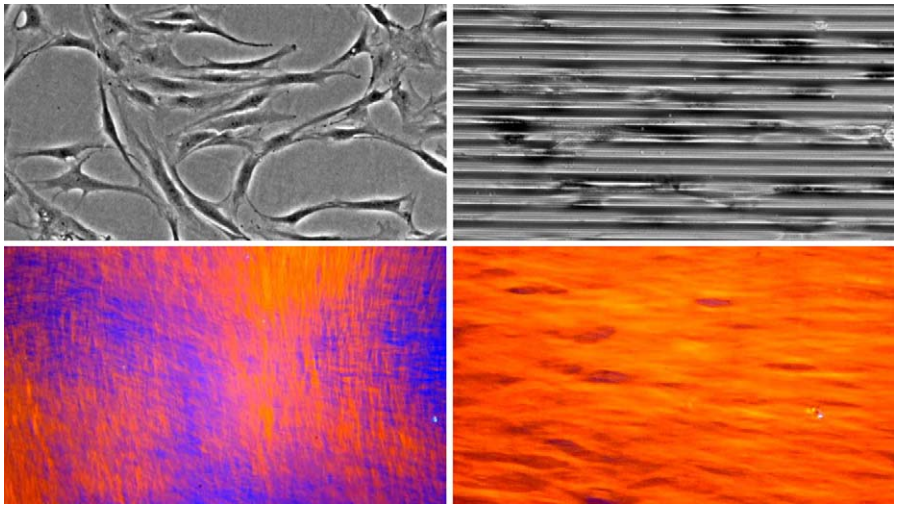

Fig. 6. Randomly aligned cells cultured on a smooth dish (upper left). Aligned cells culture on dish etched with microgroove (upper right). Randomly aligned matrix produced by cells cultured on a smooth dish (lower left). Aligned matrix produced by cells culture on dish etched with microgrooves (lower right) (Wang et al., 2003).

the forces in ligaments and ligament grafts, i.e. buckle transducers, implantable transducers, transducers at ligament insertion sites, linkage systems, cutting studies, etc. (Butler et al., 1980; Holden et al., 1994; Hollis et al., 1991; Lewis et al., 1982; Markolf et al., 1990).

In general, translations are described as proximal–distal ($d_{.PD}$), medial–lateral ($d_{.ML}$), and anterior–posterior ($d_{.AP}$) translations, while rotations are referred to as internal–external rotation ($\Theta_{.IE}$), flexion–extension ($\Theta_{.FE}$), and varus–valgus ($\Theta_{.VV}$) rotation. These motions are based on three anatomical axes: the axis of the tibial shaft, the axis defined by the femoral insertion sites of the collateral ligaments, and the floating axis perpendicular to these two axes (Fig. 7) (Chao, 1980; Grood and Suntay, 1983).

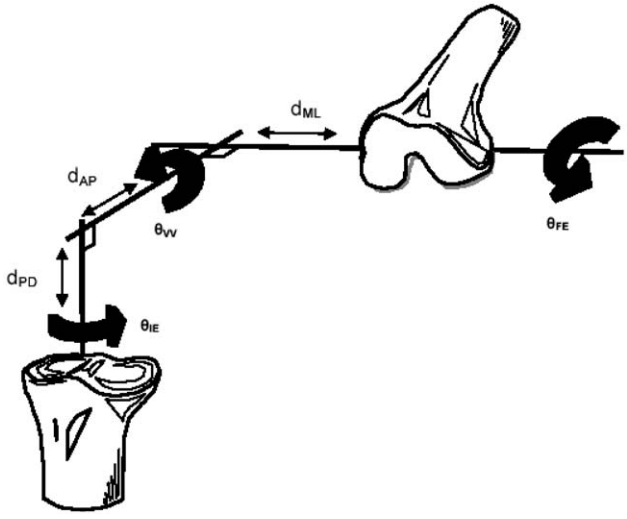

Fig. 7. Diagram detailing the joint motion description and the translations and rotations for its three anatomical axes (adapted from Woo et al., 1994, permission requested from Knee Surgery).

It is very difficult to accurately control and reproduce knee motion in all 6 DOFs. Therefore, previous studies have been forced to constrain some of the degrees of freedom of knee motion. Thus, data may not reflect the true function of the knee ligaments. For example, it was found that, when a valgus stress is applied to the knee, the ACL, rather than the MCL, is the primary restraint to varus–valgus rotation when the knee was allowed five DOF of motion (angle of knee flexion was fixed) (Markolf et al., 1976). However, if the anterior–posterior translation and axial tibial rotations were restricted (i.e. three DOF), then the role of the MCL, and not the ACL was more dominant. It can be difficult to compare results between different studies as the degrees of freedom permitted during testing can have a significant effect on the outcome (Ahmed et al., 1992, 1987; An et al., 1990; Barry and Ahmed, 1986; Lewis et al., 1989, 1982).

About a decade ago, our research center developed a robotic/universal force moment sensor (UFS) testing system (Fig. 8) for the purpose of controlling and reproducing the multiple degrees of freedom of knee motion. This novel testing system has been used to assess the function of the ACL and ACL grafts as well as that of other ligaments and joints. To date, as many as 65 studies have been published using this technology (Woo et al., 1999) and many laboratories have recently adopted this technology as well (Fujie et al., 2004; Gill et al., 2003). The robotic/UFS testing system is capable of applying external loads to knees, i.e. multiple and combined loading conditions similar to those used during clinical examinations (Daniel et al., 1985).

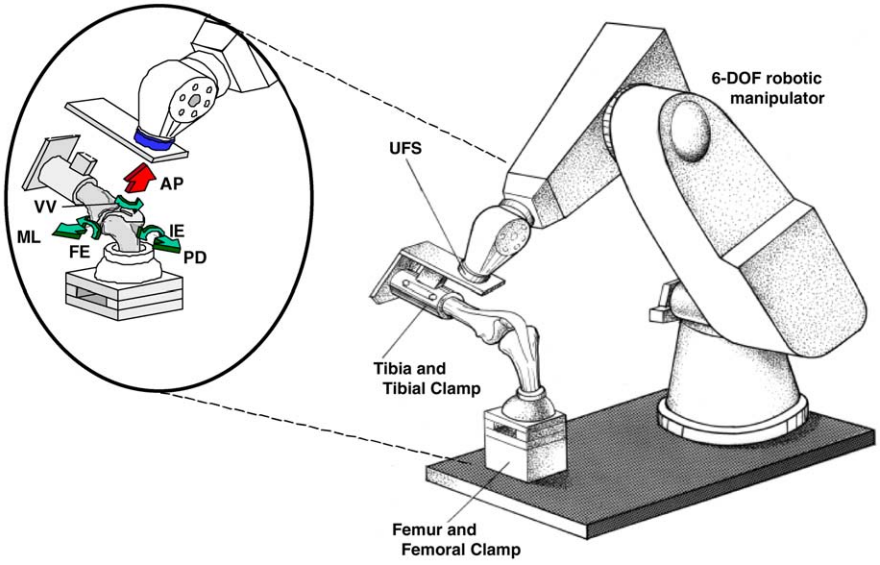

Fig. 8. Schematic drawing illustrating the six degrees of freedom of motion of the human knee joint.

Additionally, the robotic/UFS testing system can quantitatively measure the in-situ forces in ligaments and replacement grafts. The motions of the intact, ligament deficient, and reconstructed knee can be obtained with respect to the same reference position (Ma et al., 2000). Most importantly, this advanced methodology has the advantage of collecting experimental data from the *same* cadaveric knee specimen under different experimental conditions (such as ACL intact, and ACL-reconstructed knee states), thus reducing the effect of interspecimen variation and significantly increasing the statistical power of the data through the use of repeated-measures analysis of variance for data analysis. In other words, even with a large standard deviation, statistical significance can be demonstrated as long as the change in data is consistent between each experimental condition.

The robotic/UFS testing system can operate in both force and position control modes. While operating in force control mode, the robot applies a predetermined external load to the specimen and the corresponding kinematics can be obtained. Alternatively, the robotic/UFS testing system can operate under position control mode by moving the specimen along a previously recorded motion path and the UFS records a new set of force and moment data. The UFS is capable of measuring three forces and three moments about and along a Cartesian coordinate system fixed with respect to the sensor. These forces and moments are then translated to a point of application at the joint center in order to determine the magnitude and direction of the applied external loads (Fujie et al., 1995). Since the path of motion can be precisely repeated with the robotic/UFS testing system, the in situ force in a ligament can be calculated by determining the changes in forces after cutting a ligament, based on the principle of superposition (Rudy et al., 1996).

Using this testing system, we have found that the two anatomical bundles of the ACL (i.e. the anteromedial (AM) and posterolateral (PL) bundles) each function individually even under the simplest loading condition such as an anterior tibial load applied to the knee (Fig. 9) (Sakane et al., 1997). We have also learned that the ACL can resist anterior tibial translation in response to a combined internal tibial torque and valgus torque; therefore, in response to this combined rotatory load, the knee undergoes anterior tibial subluxation when the ligament is deficient (Fukuda et al., 2003; Gabriel et al., 2004).

Currently, the majority of ACL reconstruction procedures are performed by utilizing either the ipsilateral bone-patellar tendon-bone or hamstring tendon grafts. A study from our research center comparing these two graft choices indicates that under anterior tibial loads, both grafts were successful in restraining anterior tibial translation when compared to

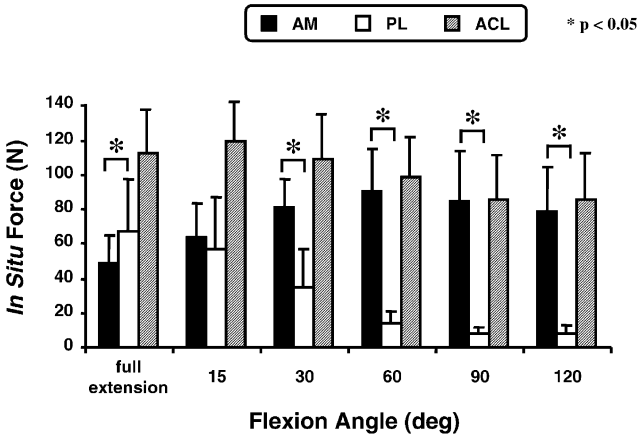

Fig. 9. Magnitude of the in situ forces in the intact anterior cruciate ligament (ACL), anteromedial (AM) bundle and posterolateral (PL) bundle under 134N of applied anterior tibial load (adapted from Gabriel et al. (Gabriel et al., 2004)).

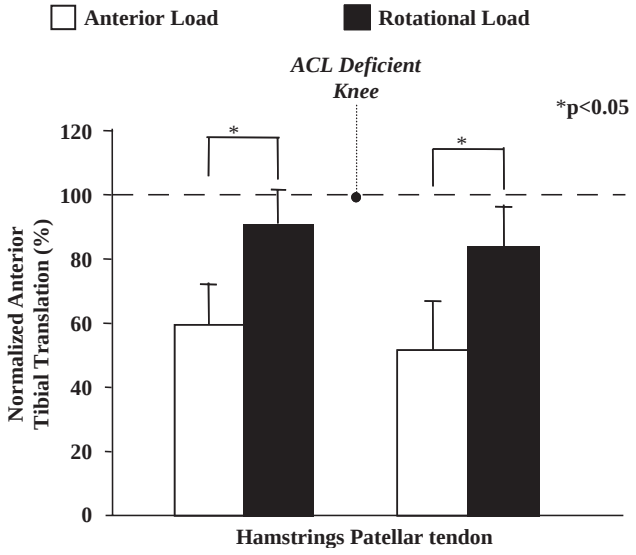

Fig. 10. Anterior tibial translation (mean ± SD) in the reconstructed knee (normalized to the deficient knee) in response to anterior tibial load and combined rotational load at 30° of knee flexion ($n = 12$) (permission requested from (Woo et al., 2002)).

that of the ACL-deficient knee. However, under rotatory loads, neither replacement graft was able to reduce the anterior tibial translation significantly when compared to that of the ACL-deficient knee (Fig. 10; note that the black bars approach the dashed lined which represents an ACL deficient knee). Although both grafts were able to restore the in situ forces in the intact ACL under anterior tibial loads, neither were successful in restoring the in situ forces to those experienced by the knee with an intact ACL under rotatory loads (Fig. 11; in this figure the dashed line represents the in-situ force in the intact ACL).

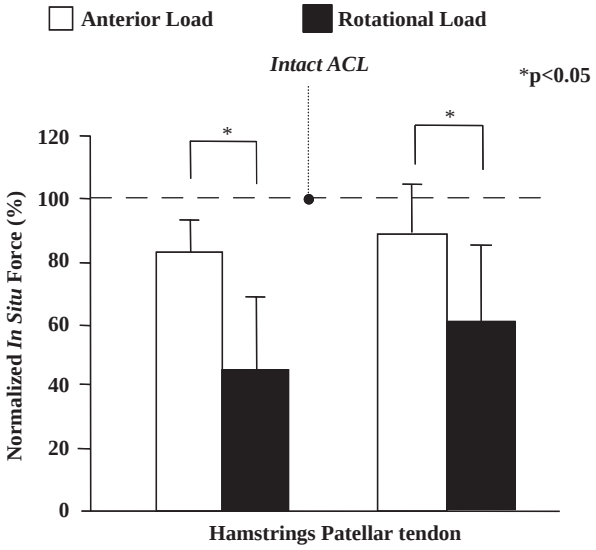

Fig. 11. In situ force in the replacement grafts (normalized to the force in the intact ACL) in response to anterior tibial load and combined rotational load at 15° of knee flexion ($n = 12$) (permission requested from (Woo et al., 2002)).

Based on the anatomy of the ACL, it appears that common reconstructive procedures place the ACL grafts too close to the central axis of the tibia and femur, thus making them inadequate for resisting rotatory loads (Kanamori et al., 2000; Woo et al., 2002; Yagi et al., 2002). Therefore, more lateral graft placement that is closer to the femoral insertion of the PL bundle has been examined (Kanamori et al., 2000; Woo et al., 2002). A series of studies from our research center were done to find biomechanical solutions to this issue. First, it was found that a more laterally placed graft yielded better results, especially in resisting rotatory loads, even though graft placement had little effect in resisting the anterior tibial load.

Second, an anatomic double bundle reconstruction that replicates both the AM and PL bundle yielded results that were closer to that of the intact knee when compared to a single-bundle reconstruction (Yagi et al., 2002). These data have generated much clinical interest, and surgeons, first in Asia and then in Europe, have recently begun to adopt the anatomic double bundle reconstruction. Likewise, some surgeons in America have recently begun to advocate this approach.

7.2. Graft incorporation and remodeling

Early graft incorporation and remodeling of ACL grafts are essential to the success of ACL reconstruction. This process is dependent on the cellular response to the mechanical forces applied to the graft during the healing process and the amount of graft motion within the bone tunnel. Studies have demonstrated that the time for complete graft incorporation differs significantly between different interfaces, i.e. bone to bone or tendon to bone interfaces (Grana et al., 1994; Jackson et al., 1993; Singhatat et al., 2002; Weiler et al., 2002; Weiler et al., 2002). ACL reconstructions in a goat model using bone-patellar tendon grafts offer the ability to study bone to bone healing and soft-tissue to bone healing in the same animal. Histological evaluations from 3 to 6 weeks revealed progressive and complete incorporation of the bone block in the femoral tunnel, but only partial incorporation of the tendinous part of the graft in the tibial tunnel.

In recent years, studies have aimed to enhance the rate of integration of tendon-bone interfaces during early graft incorporation that would permit an earlier and more aggressive postoperative rehabilitation (Chen et al., 2002). The use of bone morphogenic protein-2 (BMP-2) has shown some potential (Martinek et al., 2002) in both canine and rabbit models. The interface between the tendon graft treated with adenoviral-BMP-2-vector (AdBMP-2) and the bone was similar to the insertion of a normal ACL. Also, the stiffness and ultimate load of the graft complexes were significantly better for the AdBMP-2 treated grafts than for the control grafts at eight weeks after surgery. Biological scaffolds, i.e. periosteum, have also been explored as an interface between tendon and bone has shown some success (Chen et al., 2002). All these results suggest an exciting potential for clinical application. However, there remains a need to identify the ideal growth factor and its dosage, as well as to consider any potential safety concerns of using biological factors to augment bone-tendon healing.

Concerns of graft-tunnel motion have led to studies to evaluate the amount of motion that occurs in a hamstring reconstruction using a titanium button and polyester tape construct (Hoher et al., 1999). Shortening the tape length from 35 to 15 mm could significantly reduce the motion by 33%, as 90% of this elongation resulted from the tape. A further study revealed that a graft secured by a biodegradable interference screw can shorten the effective length of the graft, thus minimizing the amount of graft-tunnel motion (Tsuda et al., 2002).

In addition, it should also be noted that other factors including initial fixation strength (Kousa et al., 2003a, b), tibial position during fixation (Hoher et al., 2001), and initial graft tension (Abramowitch et al., 2003b; Yasuda et al., 1997) may influence graft tunnel motion, the biological integration of the graft into the bone tunnel, and ultimately ACL function.

8. Future directions

During the past three decades, significant advances have been made in characterizing the biomechanical and biochemical properties of knee ligaments as an

individual component as well as determining the contribution of ligaments to joint kinematics and function. The tensile and viscoelastic properties of ligaments, together with experimental and biologic factors, have all helped to move the field forward. Further, significant knowledge on the healing process and replacement of ligaments after rupture can serve as the basis for evaluating the effects of repair and reconstruction.

This is indeed an exciting period for ligament research. The new field of tissue engineering has offered many possibilities (e.g. growth factors, gene transfer/gene therapy, and biological scaffolds) to examine the molecular and cellular response that can enhance the healing tissue with improved properties. In our research center, we believe a tissue engineered SIS scaffold can further enhance the healing of ligaments. It is further possible to improve this bioscaffold by seeding it with ligament fibroblasts and then applying mechanical conditioning to help the alignment of the collagen fibers within the scaffold. Eventually, a combination of seeding cells on a bioscaffold that is conditioned with the ideal combination of mechanical stimuli and by the roles of AS-ODNs for types V and III collagens could be found to improve healing of ligaments. Indeed, there is still a long way to go to translate cell responses to in vivo situations and eventually to clinical application. As the biology is so complex, it is evident that an approach that involves the seamless integration of the fields of biomechanics with other biological sciences is a necessity. With that, improved outcomes in the process of ligament healing may be expected. Furthermore, what is learned can be extended to other ligaments and tendons that do not have the healing capability.

In terms of ligament reconstruction by replacement grafts, it is time to move our focus towards in vivo situations in order to optimize rehabilitation protocols and provide athletes with an earlier return to sports. While the robotic/UFS testing system has enabled us to better understand the function of the knee ligaments and has shown the road map to better ACL reconstruction, important questions that remain include the identification of the mechanism of ACL and other ligament injuries, the best reconstruction procedures, and the time course of healing and remodeling of the grafts. Therefore, in vivo kinematics data will need to be collected and then reproduced on cadaveric knees utilizing the robotic/UFS testing system (Fig. 12). Major efforts have been made in our research center on the reproducibility of data when matching cadaveric knees to groups of human subjects with similar knee laxity. Thus, an estimate of the forces in the ACL during in vivo activities may be obtained from cadaveric knees using this novel methodology. Moreover, in vivo kinematics can be integrated into computational models, and the in situ forces in ligaments during in vivo activities can be determined. Once such a model is validated through experimentation, it will be possible to use the computational model to study complex external loading conditions. These computational models can also be used to develop a database containing the in situ forces in ligaments, as well as the stress and strain data for patients of different ages, genders, and sizes. Furthermore, this technology and methodology can be

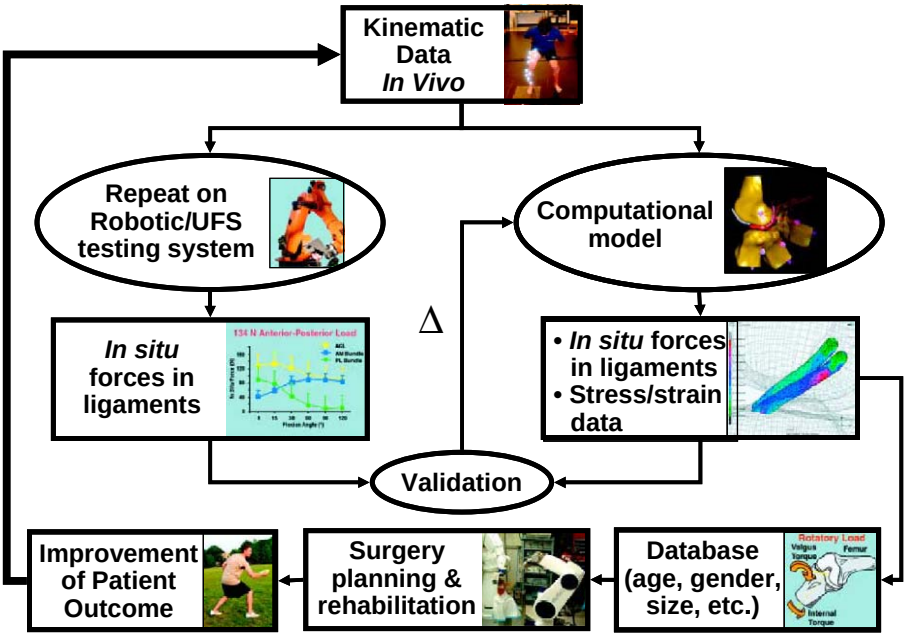

Fig. 12. Flow chart showing the utilization of in vivo kinematics data to drive experimental and computational methodologies leading to improved patient outcome.

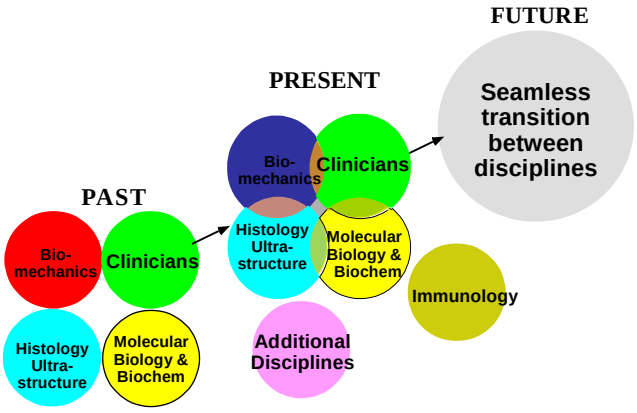

Fig. 13. Timeline of the interactions between the multiple disciplines involved in the study of tendon and ligament biomechanics, with the future holding the potential for a seamless transition between disciplines.

extended to study ligament and tendon injuries that occur frequently, such as those in the shoulder.

Ligament research has, from a biological and biomechanical viewpoint, reached an exciting time where the development of improved methods of treating ligament injuries can be a reality. Obviously, it will require an interdisciplinary and multidisciplinary research team to accomplish these goals. Biologists, biochemists, clinicians, bioengineers and other scientific experts (i.e. mathematicians, statisticians and immunologists) will work together in a seamless manner with no walls between these disciplines (Fig. 13). With that, patients will be able to completely recover from their ligament injuries and resume both normal daily activities as well as sports.

Acknowledgements

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