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Contribution of Biomechanics to Management of Ligament and Tendon Injuries

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Abstract: The contribution of biomechanics to the advancement of management of ligament and tendon injuries has been significant. Thanks to Professor Y.C. Fung's writing and guidance, our field of research has done fundamental work on anatomy and biology of ligaments and tendons, developed methods to accurately determine mechanical properties, identified various experimental factors which could change the outcome measurements as well as examined biological factors that change tissue properties in-vivo. Professor Fung also gave us his quasi-linear viscoelastic theory for soft tissues so that the time and history dependent properties of ligaments and tendons could be properly described.

We have further adopted Professor Fung's eight steps on methods of approach for biomechanical investigation to understand as well as enhance the treatment of ligament and tendon injuries during work or sports related activities. Examples on how to better treat the tears of the medial collateral ligament of the knee, as well as how to improve reconstruction procedures for the anterior cruciate ligament are presented in detail. Currently the use of functional tissue engineering for ligament and tendon healing is a topic of great interest. Here the use of biological scaffolds, such as porcine small intestinal submucosa, has shown promise.

For the last 35 to 40 years, the field of biomechanics has made great strides in the treatment of ligament and tendon injuries, and many patients have benefited. The future is even brighter because of what has been done properly in the past. Exciting advances can be made in the field of tissue engineering through novel in-vitro culture and bioscaffold fabrication techniques. Recent technology can also allow the collection of in-vivo data so that ligament and tendon injuries can be better understood. Yet, solving new and more complex problems must still follow the stepwise methods of approach as taught by Professor Fung.

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1 Historical Perspective

It is a great honor to be invited to write this review paper in this special issue to commemorate the celebratory occasion of Professor Yuan-Cheng Fung's 90^{th} birthday. We are privileged to be selected to discuss the contribution of biomechanics to the advancement of management of ligament and tendon injuries. The readers will find that Professor Fung's writing and teaching have had a tremendous influence on this field of research, from fundamental studies of translation to the clinical arena. The senior author (SW) had the great fortune of working in close proximity with Professor Fung at the University of California San Diego for 20 years, and has followed his philosophy and lessons, and in turn, has taught his students how to perform research properly. In this review paper, we trust that the reader will find the published works of biology and biomechanics of ligaments and tendons carry Professor Fung's signature.

In Professor Fung's book, entitled *Biomechanics: Mechanical Properties of Living Tissues* (Springer-Verlag, 1981), he had clearly outlined the eight essential steps to investigate problems on biomechanics (1). These steps are:

1. to study the morphology, anatomy and histology of tissues and organs in order to know

your geometric configuration,

2. to determine the mechanical properties of the materials (or tissues),
3. to use fundamental laws of physics (conservation of mass, conservation of momentum, conservation of energy, Maxwell's equations and so on), and the constitutive equations of material to derive the governing equations,
4. to understand the environment in which tissues and organs work in order to obtain meaningful boundary conditions,
5. to solve the problems analytically (or numerically),
6. to perform physiological experiments that will test the solutions,
7. to compare experimental results with the corresponding theoretical ones in order to determine whether the hypotheses made in the theories are justified, and finally,
8. to explore the practical application of the theory and results of experiments.

Professor Fung also emphasized that the most serious frustration to a biomechanics worker is usually a lack of information on constitutive equations of living tissues (i.e., step # 3). He also taught us that interaction between the theory and experiment must be an iterative one.

In this review article, we will demonstrate that the pursuit of knowledge on ligaments and tendons has followed the aforementioned methods of approach. After studies on basic anatomy and biology of tendons and ligaments, significant efforts have been given to the determination of the mechanical properties of various ligaments and tendons. In this regard, the use of video dimensional analyzer systems to measure tissue strain, as well as new methods to measure their cross sectional area, were all originated in Professor Fung's laboratory. Armed with those improved methods, their stress-strain relationships could be accurately determined. The effects of specimen orientation, strain rate, temperature, and storage on tissue properties were elucidated. In addition, the biological factors, such as maturation and aging, different anatomical location of ligaments and tendons as well as their homeostatic responses in-vivo, were found. Professor Fung also gave us his quasi-linear viscoelastic theory for soft tissues so that the time and history dependent properties of ligaments and tendons could be appropriately described. Investigators in our field have gone ahead and solved a number of boundary value problems including analysis of anisotropic behavior of ligaments and tendons, finite element numerical methods, forward dynamic and inverse dynamics problems, and had their findings validated by experiments.

The field has further adopted Professor Fung's methods of approach on biomechanics to enhance the clinical management of ligament and tendon injuries so that patients could return to work and sports related activities. Examples will include how laboratory results have been directly translated to improve treatment of tears of the medial collateral ligament (MCL), as well as how to improve reconstruction of anterior cruciate ligament (ACL) of the knee. Tissue engineering, another term coined by Professor Fung, has generated well deserved momentum and has brought biologists and bioengineers to work together. Novel and innovative approaches, including genetic and cellular therapies, as well as the use of biological scaffolds, to improve the healing of (and possibly regenerating) ligaments and tendons are ongoing, and we will cover them in this review article.

For the last 40 years, thanks to Professor Fung's teaching and philosophy, the field of biomechanics has made great strides on the understanding of ligaments and tendons. The future of this area of research is indeed much brighter because of what was done properly in the past. It also presents itself as an exciting time as we are able to move from in-vitro and animal experiments into in-vivo human applications. Yet, one must be reminded that the approach of solving new and more complex problems must always follow the methods of approach as outlined by Professor Fung and remember his advice on the iterative aspect of theory and experiment.

1.1 Basic anatomy and biology of normal ligaments and tendons

Ligaments and tendons are composed of closely packed collagen fiber bundles oriented in a parallel fashion to provide stability as well as to move synovial (diarthrodial) joints in the musculoskeletal system. In the human knee, four major ligaments transmit forces and facilitate joint movement (Figure 1). The ACL and the posterior cruciate ligament (PCL), which are located within the joint space surrounded by synovial fluid, restrain the anterior and posterior tibial displacement with respect to the femur, and more importantly, maintain the rotational stability of the knee. The other ligamentous structures include the MCL, lateral collateral ligament (LCL), posteromedial/lateral complexes, and joint capsule and serve collectively to stabilize the knee. Additional ligaments, such as the anterior meniscofemoral ligament (i.e. ligamentum Humphrey) the posterior meniscofemoral ligament (i.e. ligamentum Wrisberg) (1) are found in some people.

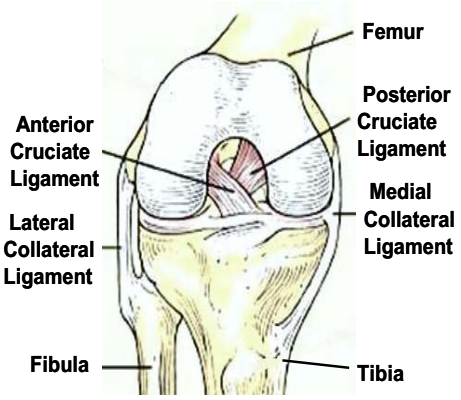

Figure 1: The major knee ligaments of the knee: the anterior cruciate ligament (ACL), posterior cruciate ligament (PCL), medial collateral ligament (MCL), and lateral collateral ligament (LCL) (permission requested from (2)).

Generally, ligaments and tendons have a hierarchy of fibrillar arrangement organized into a structure composed of fibrils, fibers, subfascicular units, fasciculi, and the tissue itself. Additionally, it may be composed into more than one large bundle, as in the cruciate ligaments of the knee. Ligaments transmit forces between bones (forming a bone-ligament-bone complex), while tendons transmit muscle forces to bone for skeletal control (forming a muscle-tendon-bone complex). Tendon and ligament insertions to bone are functionally adapted to distribute and dissipate forces by transmitting them through the fibrocartilage to bone. The bony attachments of ligaments and tendons are complex and vary considerably.

Ligaments and tendons consist of water (65% to 70% of the total weight), collagen, elastin, proteoglycans, and glycolipids. They are relatively hypovascular (2) and hypocellular, with <5% of the total volume occupied by cells (predominantly fibroblast) (5). Roughly 70% to 80% of the dry weight of normal tendon or ligament is composed of type I collagen, which is primarily responsible for the stiffness and strength of the tissue. Other types of collagen, including types III, V, X, XI, and XII, also exist but in minor amounts. Type V collagen is believed to exist in association with type I collagen and serves as a regulator of collagen fibril diameter (6), whereas type III collagen is needed for ligament and tendon repair (7). Variations in the concentrations of these basic constituents plus their anatomy lead to a diverse array of biomechanical behaviors of knee ligaments and tendons that are suitable for their respective functions.

2 Application of Biomechanics to Understand Tensile Properties of Ligaments & Tendons

2.1 Determination of Mechanical Properties

To measure the biomechanical properties of ligaments and tendons, a uniaxial tensile test of a bone-ligament-bone complex or muscle-tendon-bone complex is usually done because the function of these tissues is to transmit tensile loads in-vivo. These tests result in a load-elongation curve (describing their structural properties) that is nonlinear with a slope that increases with increasing elongation. This unique relationship also enables these tissues to maintain smooth movement of joints at loads for normal physiologic ac-

tivities while restraining excessive joint displacements under high loads. With additional cross-sectional area and strain measurements, a stress-strain curve representing the mechanical properties of the ligament or tendon substance can also be obtained. Nevertheless, there have been many challenges presented in the determination of the biomechanical properties of ligaments and tendons, due to their deformability and complex, irregular geometry. Thus, the readers should be aware that the data reported in the literature do have large discrepancies.

2.1.1 Measurement of stress and strain

Special efforts are needed to accurately measure the mechanical properties of ligaments and tendons. Many techniques have been developed, and these can be separated into either contact or non-contact methods (8). Contact methods are generally easier to use, but can introduce errors (9). Our laboratory has developed a laser micrometer system to measure both the cross-sectional area and the shape of convex soft tissues that has been shown to be highly accurate and precise (9). Recently, a more advanced laser reflectance system has also been developed by us to measure cross-sectional shape and area of soft tissues with concave surfaces (8).

An accurate, experimental measurement of tissue strain of ligaments and tendons has been the use of video tracking systems (as pioneered in Dr. Fung's laboratory) to avoid possible errors of elongation from contribution of their connecting structures. With two or more reference markers placed on the surface of the tendon or ligament substance (by means of an elastin stain (10) or reflective tape (11)) to serve as gauge lengths, a video or other camera system could record the motion of the markers during tensile stretch and percentage strain of the tissue could be calculated (12).

The range of mechanical properties of various ligaments and tendons is huge as they are designed specifically to function in different joints (Table 1). For the human MCL, the tangent modulus and the ultimate tensile strength were found to be 332.2 $\pm$ 58.3 MPa and 38.6 $\pm$ 4.8 MPa, respectively (13). In comparison, values for the inferior glenohumeral ligament ranged from 5 to 42 MPa and 1 to 6 MPa, respectively, approximately 10% of those for the MCL (14). Therefore, the large range of motion and flexibility of the glenohumeral joint requires that the properties of its ligaments and tendons suit its functional demands.

2.1.2 Contribution of other experimental factors

Experimental factors, such as specimen orientation or strain rate, can largely impact the biomechanical properties of ligaments and tendons (26, 27). For example, the structural properties for the human femur-ACL-tibia complex (FATC) tested in an anatomic orientation, which maintained the natural insertion angles of the ACL, were significantly higher than those tested in a tibial orientation (26). For example in young donors, the ultimate load of specimens in the anatomic orientation (2160 $\pm$ 167 N) was 35% higher than those in the tibial orientation (1604 $\pm$ 157 N). The tibial orientation also resulted in more insertion site failures. Thus, the anatomic orientation allowed for a smooth transition of load from bone to ligament as well as a more uniform load distribution within the ligament.

Considerable attention has been given to the effects of the strain rate on the mechanical properties of soft tissues. Professor Fung's work on the rabbit skin, rabbit papillary muscle of the right ventricle, canine ureter, rabbit mesentery, and so on (1, 28-31) had found that these tissues are generally insensitive to strain rate, i.e. with only a 1- to 2-fold change in stress for a given strain over 1000-fold change in strain rate. For the mechanical properties of ligaments and tendons, our laboratory studied the rabbit MCL, ACL, and patellar tendon (PT) (27, 32, 33). For skeletally immature animals, there were some strain rate sensitivity ($\sim$50% to 100% increases for some parameters); whereas in skeletally mature animals, there were none over a range of four and a half decades of strain rates.

Environmental conditions, including temperature and hydration, are also important considerations when testing ligaments and tendons. For example, in the canine femur-MCL-tibia complex

Table 1: Mechanical properties of human ligaments and tendons

Tissue	Modulus (MPa)	Ultimate Tensile Strength (MPa)	Ultimate Strain (%)	Source
Knee				
Medial Collateral Ligament	332 ± 58	39 ± 5	17 ± 2	(13)
Anterior Cruciate Ligament	65-447	13-46	15-44	(15-17)
Posterior Cruciate Ligament	150-447	30-36	11-19	(15, 18, 19)
Patellar Tendon	504-680	54-65	12-15	(15, 20, 21)
Lower extremity				
Gracilis Tendon	590-1734	111-112	7-19.4	(20, 22)
Semitendinosus Tendon	540-1081	89-124	8-23	(20, 22)
Achilles Tendon	819 ± 208	79 ± 22	9 ± 2	(23)
Shoulder				
Inferior Glenohumeral Ligament	5-42	1-6	8-33	(14, 24)
Posterior Glenohumeral Capsule	10-32	2-5	22-23	(25)

(FMTC), during cyclic elongation, peak load at the first cycle increased linearly as the temperature declined ($\sim$0.75 N/$^{\circ}$C) (34). In human PTs, a significantly higher tangent modulus and tensile strength were found when tested in a temperature-controlled saline bath (307 $\pm$ 17 MPa and 43.7 $\pm$ 3.9 MPa, respectively) than when tested in air with dripping saline (191 $\pm$ 16 MPa and 30.6 $\pm$ 2.6 MPa, respectively) (35).

Due to complex biomechanical testing methodologies, specimens are often stored by freezing, which may affect the mechanical properties of these tissues (10, 36, 37). A study using the rabbit MCL model found no significant differences between fresh and frozen samples in the mechanical properties of the rabbit MCL substance and of the structural properties of the FMTC following 3 months of frozen storage (10). Further, a follow-up study revealed that an additional freeze-thaw cycle did not significantly alter these biomechanical properties, including the viscoelastic behavior of the MCL (36). However, care still must be taken in preparing the tissue sample before and after freezing in order to protect the sample from dehydration. The experimental factors outlined in this section can have profound effects on the outcome of soft tissue behavior and are of particular importance in explaining why findings may differ from study to study.

2.2 Contribution of Biological Factors

Ligaments and tendons are subjected to morphological, biochemical, and biomechanical changes in-vivo that are specific to their anatomical location, as well as the degree of skeletal maturation, age, and activity level of the animal. Skeletal maturity has been shown to play a major role in the biomechanical properties of tendons, ligaments, and their insertions based on animal models. For the New Zealand white rabbit, the structural properties of its FMTC and mechanical properties of its MCL substance were shown to increase dramatically from 6 to 12 months of age (38, 39) and remain relatively constant thereafter. Interestingly, all skeletally immature FMTCs failed by tibial avulsion; whereas, the majority of skeletally mature animals failed at the tissue substance indicating that there is an asynchronous maturation process between the bone-ligament-bone complex and ligament midsubstance (39) (Figure 2).

The properties of ligaments and tendons with advancing age and changes in the activity level and/or the physical condition may also change (40). For the human FATC, the mean stiffness and ultimate load of younger specimens (22-41 years) was 1.3 and 3.3 times higher, respectively, than that of older specimens (60-97 years) (26). Further, older specimens had a higher incidence of midsubstance failure (86% vs. only 57% for younger specimens), suggesting that the

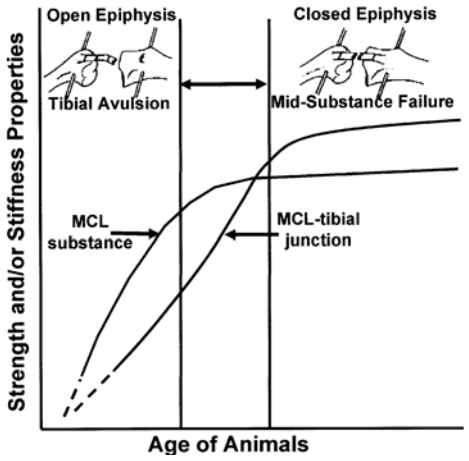

Figure 2: A schematic diagram depicting the relationship between failure mode and age, hypothesizing the asynchronous rates of maturation between the bone–ligament–bone complex and the ligament substance (permission requested from (39)).

bones weakened faster than the ligaments. Similar results have been observed in the shoulder ligaments and joint capsule (14, 41). On the other hand, there was little change in the load–elongation curve of the rabbit FMTC between skeletal maturity and the onset of senescence (27), illustrating that age-dependent changes are not uniform between ligaments.

Connective tissues are known to respond to motion and stress. As many treatments of orthopaedic problems had involved a period of immobilization followed by rehabilitation, studies using rabbit hind limbs have shown how these protocols would negatively decrease the structural properties of the FMTC and mechanical properties of the MCL tissue with a few weeks of immobilization (42). Remobilization could reverse these negative changes, but would require up to one year of remobilization for their properties of the ligament to return to normal levels (43). Similar results were found for the FATC of primates and rabbits (44, 45). Exercise and training, on the other hand, showed only marginal increases in the structural properties of ligaments and tendons, including the MCL and extensor and flexor tendons of the hand (46-48). For example, only a 14% increase in linear stiffness was seen for the porcine FMTC after one year of exercise with minimal changes in the mechanical properties of the ligament and tendon substance. Based on these results and related literature, a highly nonlinear representation of the relationship between different levels of stress and ligament and tendon properties as depicted in Figure 3 has been proposed. 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, the positive changes following long-term exercise and training are much more moderate.

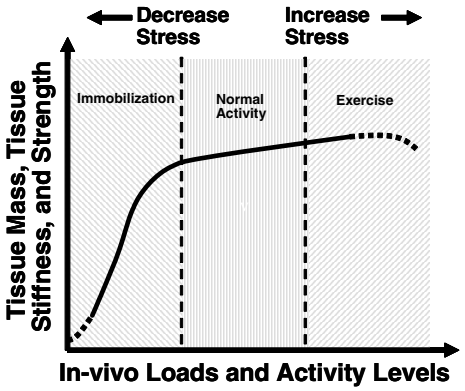

Figure 3: A schematic diagram describing the homeostatic responses of ligaments and tendons in response to different levels of stress and motion (permission requested from (43)).

2.3 Anisotropic Properties

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 (13). 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, T, can then be written as,

$$T = 2\{(W_1 + \mathrm{I}_1 W_2)\mathrm{B} - \mathrm{W}_2 B^2\} + \lambda W_\lambda a \otimes a + \rho 1, \quad (3)$$

where, 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, 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 (Figure 4). The longitudinal specimens displayed a tangent modulus of 332.2 $\pm$ 58.3 MPa and a tensile strength of 38.6 $\pm$ 4.8 MPa; whereas, the transverse specimens were 11.0 $\pm$ 0.9 MPa and 1.7 $\pm$ 0.5 MPa respectively, a difference of about an order of magnitude (13).

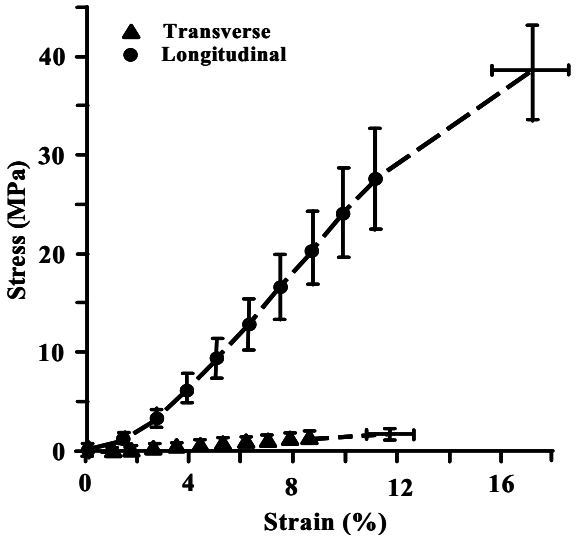

Figure 4: Stress-strain curves for human MCLs longitudinal and transverse to the collagen fiber direction (permission requested from (13)).

2.4 Viscoelastic Properties of Ligaments and Tendons

The complex interactions of collagen with elastin, proteoglycans, ground substance, and water result in the time- and history-dependent viscoelastic behaviors of ligaments and tendons. In response to various tensile loading protocols, ligaments and tendons exhibit hysteresis (i.e. internal energy dissipation), creep, and stress relaxation. The following is a comprehensive summary of the theories to describe these properties.

2.4.1 The Quasi-Linear Viscoelastic Theory

The quasi-linear viscoelastic (QLV) theory developed by Professor Fung (1) is one of the most successful models to describe the viscoelastic properties of soft tissues (49-54). 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:

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

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

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

Using Fung's generalized relaxation function based on the assumption of a continuous relaxation spectrum (1), the time-dependent reduced relaxation function, $G(t)$, takes the form:

$$G(t) = \frac{1+C\{E_1^{(t/\tau_2)} - E_1^{(t/\tau_1)}\}}{1+C*\ln(\tau_2^{t/\tau_1})} \quad (6)$$

where E_1 is the exponential integral, $\int_y^{\infty}\frac{e^{-z}}{z}dz$, and, C, τ_1 and τ_2 are constants with $\tau_1 \ll \tau_2$. Assuming $G(t_0)$ = 1, $\delta G(t)/\delta(t)$ = constant, and $G(\infty)$ = constant, QLV theory can be applied to a ligament during uniaxial tension tests in one dimension for small strains (<5%).

Using this approach, the QLV theory has been utilized to model a variety of ligaments and tendons, e.g. the rat tail tendon, canine, goat, and rabbit MCL, human ACL, and human PT. The readers are referred to References 55 and 56 for details (55, 56). With separate curve fitting of $\sigma^{\varepsilon}(\varepsilon)$ and $G(t)$ to the loading and relaxation portions of the experimental data, respectively, the constants of the QLV theory (A, B, C, τ_1, and τ_2) were obtained. These constants were then validated by successfully predicting the peak and valley stresses from a separate cyclic stress relaxation experiment.

The stress relaxation test is based on the assumption of a step-change in strain which is impossible to achieve experimentally. Efforts were made to account for these errors, including various normalization procedures, iterative techniques, extrapolation and deconvolution, as well as directly fitting the measured strain history (57, 58). Recently, our research center has developed an alternative approach for the QLV to be used with experiments that utilize a slower strain rate (58). By means of the Boltzmann's superposition principle, we have shown that the loading portion of a stress relaxation experiment, for $0 < t < t_0$, could be described by a linear strain history for strain rate γ:

$$\sigma(t) = \frac{AB\gamma}{1+C\ln(\tau_2/\tau_1)} \int_0^t \{1+C(E_1[(t-\tau)/\tau_2]\mathrm{E}_1[(t-\tau)/\tau_1])\}e^{B\gamma\tau}\partial\tau \tag{7}$$

The subsequent stress relaxation following a constant strain (from t_0 to $t=\infty$) can be described by a separate equation as:

$$\sigma(t) = \frac{AB\gamma}{1+C\ln(\tau_2/\tau_1)} \int_0^{t_0} \{1+C(E_1[(t-\tau)/\tau_2]\mathrm{E}_1[(t-\tau)/\tau_1])\}e^{B\gamma\tau}\partial\tau \tag{8}$$

Simultaneously curve-fitting these equations to the loading and relaxation portions of the data from a stress relaxation experiment allows the constants A, B, C, τ_1, and τ_2 to be determined (58). 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.

With this approach, the viscoelastic behavior of the goat FMTC could be described (Figure 5). The constants, especially τ_1, were improved when compared to those with a step change in strain. Further, the results were verified by the prediction of an independent cyclic strain test of the same tissue (58). More importantly, this new approach allows for the comparison of constants between tissue types and different researchers.

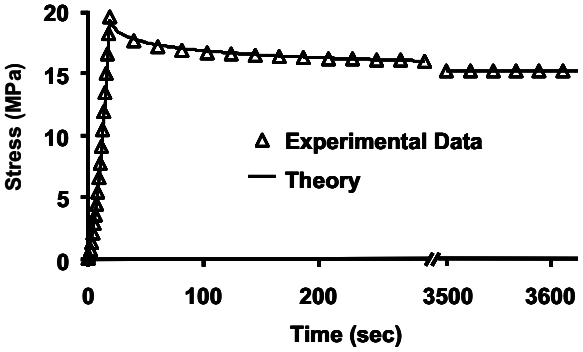

Figure 5: A typical curve fit using the new approach to experimental data obtained from a stress relaxation test of a goat FMTC (permission requested from (58)).

2.4.2 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 viscoelastic behavior is nonlinear (i.e. the rate of relaxation decreases as the level of applied strain increases up to 2.5% strain) (59, 60). However, work has demonstrated that creep and stress relaxation behaviors of the MCL likely arise from different mechanisms (61). In fact, Professor Fung in his book *Biomechanics* (2nd edition; 1993 (1)) described this phenomenon by suggesting "... creep is fundamentally more nonlinear, and perhaps does not obey the quasi-linear hypothesis."

Alternative viscoelastic models are needed to in-

clude the nonlinear behavior. In our research center we had developed a method based on the single integral finite strain (SIFS) theory to fully describe the nonlinear viscoelastic behavior of ligaments (62). The concepts of microstructural change resulting from recruitment and fading memory to ensure that more recent strain states have greater weight in determining the stress than earlier states are incorporated. The specific constitutive equation can be written as:

$$\mathbf{T} = -p\mathbf{I} + C_0\{[1+\mu I(t)]\mathbf{B}(t) - \mu\mathbf{B}^2(t)\} - (C_0 - C_\infty)\int_0^t G(t-s)\{[1+\mu I(s)]\mathbf{B}(t) - \mu\mathbf{F}(t)\mathbf{C}(s)\mathbf{F}^T(t)\}ds \quad (9)$$

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 $I(s)$ = 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. It is also important to note that when the function is restricted to one dimensional cases and additional assumptions are made, the SIFS theory reduces to the QLV theory.

The model was used for younger and older human PTs and canine MCLs (62). 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, the robustness of the SIFS theory makes it ideal for future application involving nonlinear problems with large strains.

3 Treatment of Ligament and Tendon Injuries

Ligament and tendon injuries occur frequently in work and sports related injuries, and the incidence has increased over the last few decades. It is estimated that tendon injuries account for 30% to 50% of all injuries related to sports (63), with as much as 75% of Achilles tendons ruptures occurring during sporting events (64). Similarly, the incidence of ligament injuries has also been on the rise. It is estimated that 2 in 1,000 people per year in the general population suffer from a knee ligament injury alone, while ACL and MCL injuries (200,000 and 95,000 per year in the U.S., respectively) account for as much as 90% of all sports related ligament injuries (65, 66). In addition, 2-6 for every 100 athletes injure their lateral ankle ligaments during the course of the year (67, 68). The same could be said about the shoulder as many of these injuries require treatment – both surgical and nonsurgical as well as rehabilitation (69, 70). As such, there is a tremendous need to better understand tendon and ligament injuries as well as their healing and remodeling processes in order to develop improved treatment strategies.

3.1 Healing of Knee Ligaments

3.1.1 MCL healing

Laboratory research has discovered that the injured MCL of the knee can heal spontaneously (71). Clinical studies confirmed that functional treatment produced better results to those with surgical repair (72), as post operative immobilization led 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 previously injured insertion sites (43). As a result, for the last twenty-five years the paradigm of clinical management of MCL tears has shifted from surgical repair with immobilization to functional management with early controlled motion (73, 74).

3.1.2 Phases of ligament and healing

The MCL has been an excellent experimental model to help understand the rate, quality, and composition of healing ligaments and tendons as well as treatment modalities (71, 75). It has brought better understanding of the continuous process of healing. Roughly, it can be divided into three overlapping phases (71, 76). 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, an increasingly organized extracellular matrix (ECM) formation, 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 (76).

3.2 ACL Reconstruction

Unlike the MCL, injuries to the midsubstance of the cruciate ligaments showed very limited healing capability and surgical reconstruction using replacement auto- or allografts are done. While many patients have benefited from these procedures, a significant percentage (20-25%) do have complications and unsatisfactory outcomes (77). Efforts involving biomechanics are being made in order to better our understanding of the kinematics of the knee and the function of the intact ACL and ACL replacement grafts. This approach includes measurement of six degree of freedom (DOF) knee motion and the forces in ligaments and ligament grafts via buckle transducers, implantable transducers, transducers at ligament insertion sites, cutting studies, and so on (26, 78, 79).

3.2.1 The robotic/UFS testing system

Since 1993, our research center developed a robotic/universal force moment sensor (UFS) testing system (Figure 6) for the purpose of controlling and reproducing the multiple DOF knee motion. This novel testing system has been used to assess the function of the ACL and ACL grafts as well as other ligaments and joints. To date, over 80 studies have been published using this technology (80) and many laboratories have also adopted this technology (81, 82).

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 (83). 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 (84). 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. In other words, even with a large standard deviation, statistical significance can be demonstrated as long as the change in data is consistent.

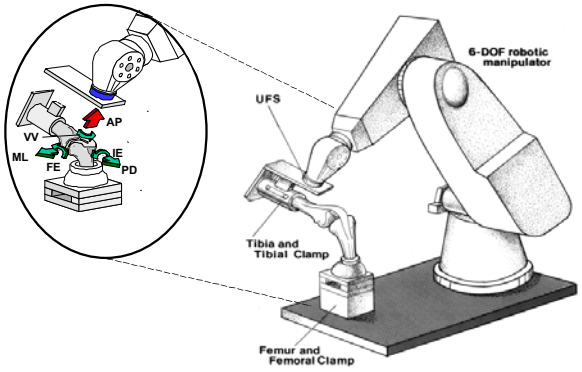

Figure 6: Schematic drawing illustrating the six degrees of freedom of motion of the human knee joint.

The 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, it can operate under position control mode by moving the specimen along a previously recorded motion path while 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 (85). Since the path of motion can be precisely repeated, 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 (86).

3.2.2 Complex function of ACL and the contribution of AM and PL bundles to knee function

Using a robotic/UFS testing system, we have found that the two anatomical bundles of the ACL (i.e. the anteromedial (AM) and posterolateral (PL) bundles) have different functions even under the simplest external loading condition applied to the knee (87). We have also learned that the ACL is the primary restraint to anterior tibial translation in response to a combined internal tibial and valgus torque. When the ACL is deficient, the knee undergoes anterior tibial subluxation in response to such a combined rotatory loading regime (88, 89). 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 revealed that under anterior tibial loads, both grafts were successful (90). However, under rotatory loads, neither replacement graft was able to reduce the anterior tibial translation significantly from those of the ACL-deficient knee.

We have developed computational finite element models to assess the complex function of the ACL and its bundles which are validated experimentally via either knee kinematics or in-situ forces determined using our robotic/UFS testing system (91, 92). First, a model of the intact knee joint was developed and able to predict anterior tibial translation to within 26% and in-situ force in the ACL to within 10% under a 100 N anterior tibial load (91). A second validated model examined the complex force and stress distribution within the AM and PL bundles of the ACL in response to an anterior tibial load (92). At full extension, both the AM and PL bundles share the load, but the PL bundle carries 43% more force than the AM. Also, the stress distribution was non-uniform, and peak stresses were seen near the femoral insertion sites (Figure 7). Current efforts aim to advance this model by accounting for the non-uniform geometry of the ACL (in which the midsubstance cross-sectional area is one-third that of the insertions) and its twisting fiber orientation.

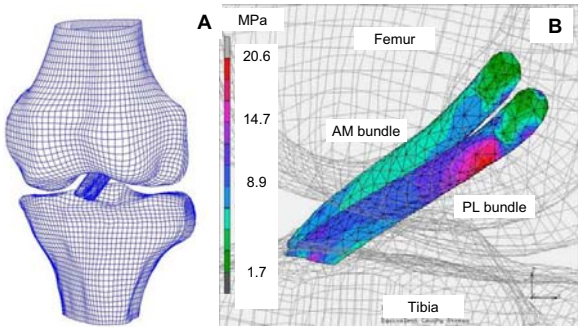

Figure 7: A) Finite element model of the knee joint and B) Cauchy stress distribution within the AM and PL bundles under a 134N anterior tibial load with the knee at full extension (lateral view). (permission requested from (92)).

3.2.3 New surgical procedure – double bundle ACL reconstruction

To investigate the issue of rotatory instability following ACL reconstruction, a series of biomechanical based experiments have been performed in our research center (90, 93, 94). First we considered, a double bundle reconstruction by replacing both the AM and PL bundles so that it would better approximate the anatomy of the ACL. It could not only restore knee kinematics and in-situ force under anterior tibial loading but also under a combined rotatory load of internal and valgus torque, as knee kinematics of the ACL with a double bundle reconstruction were closer to the intact ACL. The in situ force at 30° of flexion was 91% $\pm$ 35% of the intact ACL, compared to only 66% $\pm$ 40% for a single bundle reconstruction. A second similar study compared double bundle reconstruction to a more laterally placed single bundle reconstruction (thought to replicate the PL bundle) (95). Again, the former better restored knee kinematics and had an in-situ force in the grafts closer to normal compared to single bundle reconstruction in response to an anterior tibial load, especially at high flexion angles. Both

procedures could restore rotatory stability. These findings have provided the scientific basis for surgeons to adopt the anatomic double bundle ACL reconstruction.

3.2.4 Appropriate knee flexion for graft fixation in double bundle ACL reconstruction

With double bundle ACL reconstruction, the potential of overloading either the AM and PL graft is increased. Our research center has performed a series of experiments to find an envelope of knee flexion angles for safe graft fixation (96). It was found that the AM graft was not overloaded when the AM and PL grafts were both fixed at 30 degrees of flexion, while the PL graft was not overloaded when the AM and PL grafts were fixed at 60 degrees and full extension, respectively. Overloading of the PL graft occurred when it was fixed at 30 degrees, which is reasonable since the PL bundle is smaller and shorter than the AM bundle and functions mainly near full extension. A follow-up study further narrowed the PL graft to be fixed at 15 degrees while the AM graft was fixed at either 45 or 15 degrees of knee flexion. In this case, the in-situ forces in the AM and PL grafts were below those of the AM and PL bundles, and neither graft would be overloaded (97).

3.3 Functional Tissue Engineering to Improve Healing of Ligaments and Tendons Regeneration

Laboratory studies have demonstrated that the constituents of the healing tendons and ligaments are abnormal and remain so for a long period of time (71). For example, in the healing MCL, the mechanical properties were much inferior to those of the normal ligament and did not improve with time of healing (71, 98) (Figure 8). There was also a decrease in the number of mature collagen crosslinks, a higher ratio of type V/I collagen, and an increased amount of proteoglycans. TEM showed that collagen fibrils were homogenously small in diameter (100, 101). Concomitantly, the cross-sectional area of the healing MCL increased with time and was measured to be $2\frac{1}{2}$ times that of the normal MCL (98). This increase in quantity of tissue resulted in the recovery of the stiffness of the FMTC. Thus, the research question is whether one can improve the quality of the healing MCL tissue through novel treatment strategies. If successful, these new approaches may be further applied to ligaments and tendons that do not easily heal, such as the ACL of the knee.

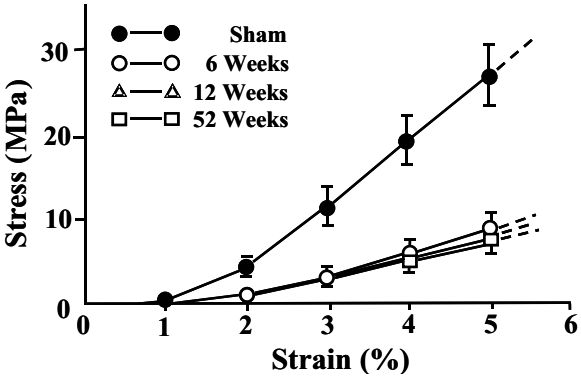

Figure 8: Stress-strain curves representing the mechanical properties of the medial collateral ligament substance for sham-operated and healing MCLs at time periods of 6 (n=6), 12 (n=6), and 52 (n=4) weeks (permission requested from (99)).

Functional tissue engineering (FTE) is a new field that combines molecular biology, biochemistry, and biomechanics and can offer novel therapeutics such as growth factors, gene transfer/gene therapy, cell therapy, biological scaffolds, and so on to improve ligament and tendon healing. The following is a brief review of these new approaches and their challenges.

3.3.1 Growth factors

Growth factors have been shown in-vitro to increase cell proliferation and migration, as well as ECM synthesis and production. In particular, fibroblast growth factor (FGF), epidermal growth factor (EGF), platelet-derived growth factor-BB (PDGF-BB), and transforming growth factor (TGF) $\beta 1$ and $\beta 2$ have been well studied (102-104). However, when extending these findings to in-vivo experiments, many contradictory results were found, suggesting that in-vivo conditions are much more complex and additional studies are needed. An area where growth factors have shown success is for quicker incorporation of an

ACL auto graft in the bone tunnel following ACL reconstruction. Using an adenoviral-bone morphogenic protein-2-vector (AdBMP-2) in both canine and rabbit models (105), significant improvement of the interface between the tendon graft and the bone as well as the stiffness and ultimate load of the graft complexes were found following treatment with AdBMP-2. Despite these exciting findings, there remains a need to identify the dosage and timing, as well as consideration of safety concerns of using these biological agents.

3.3.2 Gene therapy

Gene therapy offers a potential approach to prolong the delivery of appropriate growth factors into cells to alter protein synthesis or to induce the expression of therapeutic proteins in order to improve the properties of healing ligaments and tendons. This approach normally relies on mammalian viruses and cationic liposomes as vectors to deliver genes into host tissue, either directly or indirectly. Studies have shown that PT fibroblasts can be transduced with the LacZ marker gene both directly using an adenovirus liposomal vector (involving in-vivo injection of the delivery vector into the host tissue), or indirectly using a retrovirus (involving in-vitro transduction of host cells with the desired gene and subsequent replantation of these cultured cells in-vivo). The expression of the transferred genes persisted for 6 weeks following the application (106). In our research center, we showed that genes could be successfully transduced into MCL and ACL fibroblasts (107).

Another technique for gene transfer is antisense gene therapy which involves the binding of antisense oligodeoxynucleotides (ODN) to target DNA, thus blocking the transcription or translation of specific genes which may be excessively expressed within healing tissue. Investigators have performed direct transfer of an HVJ-liposome complex containing a labeled ODN for the protein decorin and showed type I collagen fibril formation could be inhibited (108). In our research center, we have shown the efficacy of utilizing ODNs to regulate the overproduction of collagen types III and V (109, 110).

Thus, gene therapy is an exciting avenue for future exploration. Nevertheless, it should be cautioned that this biological intervention may induce immune reaction against these antigens, (111). Further, retroviral infection of fibroblasts that could adversely affect expression of the incorporated gene (112) and repeatable delivery of the ODNs to the appropriate target are other potential problems.

3.3.3 Cell therapy

Studies on cell therapy have focused on the application of mesenchymal stem cells (MSCs) and bone marrow derived cells (BMDCs) into the ligament and tendon healing site (113, 114). Autologous marrow-derived progenitor cells embedded in collagen gels were transplanted into a gap injury in the rabbit Achilles' tendon. After 12 weeks, the mechanical properties of the healing tissue were greatly improved (114). BMDCs have also been expanded in-vitro, seeded in collagen gels and transplanted to PT defects after its removal for ACL reconstruction. In rabbits, the results showed faster rates of repairs, as the treated healing tissues significantly increased in terms of modulus and tensile strength (115, 116). Alternatively, cell therapy in combination with bioscaffolds would offer even greater possibilities.

3.3.4 Biological Scaffolds

There are a few naturally derived bioscaffolds, i.e. the porcine small intestine submucosa (SIS), urinary bladder matrix, liver derived matrix, lymphoid ECM, amniotic membrane, among others that have been successfully used for healing of soft tissues (117, 118). These scaffolds, particularly the SIS, have an attractive biological and mechanical environment to attract cells and guide the synthesis of an ECM, both of which are keys in the regeneration of these load bearing tissues. The SIS scaffold has been shown to degrade within one month in-vivo (119), and its byproducts are bioactive agents (growth factors, fibronectin, and so on) and chemoattractants for cells (120, 121). It is FDA approved and has been implanted in over 500,000 patients to date (118).

Our research center conducted a multidisciplinary study to determine the effect of SIS treatment on

MCL healing in the short- and long- term (12 and 26 weeks). It was found that its intrinsic growth factors are chemoattractants (120) that encourage tissue healing, as evidenced by a histomorphological appearance in the SIS-treated ligament that was closer to normal when compared to the non-treated ligament. The SIS also guided neo-ligament tissue formation and decreased the cross-sectional area of the healing tissue by 28%, thereby improving its mechanical properties by 33-49% (122, 123) (Figure 9). Meanwhile, a follow-up study at 6 weeks revealed that the gene expression of collagen type V as well as the small leucine-rich particles were lower in the SIS-treated group when compared to those in the non-treated group (124). As a result, the collagen type V/I ratio decreased with a concomitant formation of a heterogeneous distribution of large collagen fibril diameters at later timepoints, resulting in better healing tissue properties (Figure 10).

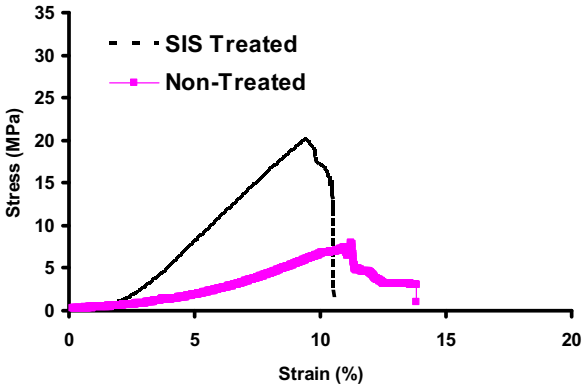

Figure 9: Typical stress–strain curves for SIS-treated and non-treated groups at 12 weeks post-injury for the healing MCL. (permission pending from 123)).

We have also conducted a second study on the use of SIS on PT healing following harvest of its central third (3 mm wide) for ACL reconstruction using a rabbit model (125). In addition to containing a variety of growth factors (121), SIS only allows cellular infiltration through its abluminal side, and thus, can prevent adhesion formation on its luminal side (126). As such, it was first hypothesized that a layer of SIS could form a barrier beneath the PT donor site with the luminal side facing the underlying fat pad in order to limit adhesion formation. Secondly, it was hypothesized that another layer of SIS applied anterior to the PT with the abluminal side facing the defect could accelerate PT healing via the bioactive degradation products of SIS (120).

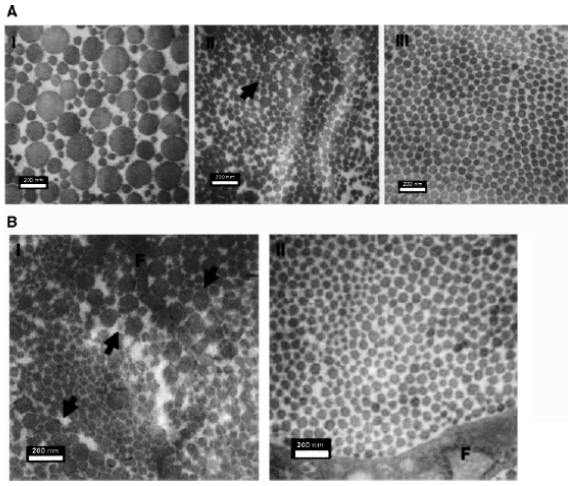

Figure 10: Transmission electron micrographs (x70,000) of collagen fibrils in (A) sham operated MCL (I), SIS-treated MCL (II) and non-treated MCL(III) at 26 weeks post-injury. The arrow indicates the appearance of large fibrils between cells in the SIS-treated MCL. (B) The TEM appearance of both large and small fibrils (heterogeneity) in the pericellular area in the SIS-treated MCL (I) and non-treated MCL (II). The arrow indicates the large fibrils surrounding a cell process. F indicates fibroblast. (permission requested from (122)).

At 12 weeks post-surgery, the PT defect filled with more healing neo-PT tissue following SIS-treatment than those without treatment, as the cross-sectional area was 68% greater. SIS-treatment also resulted in a 57% higher stiffness and 70% higher ultimate load of the healing central bone-PT-bone (BPTB) complex. Furthermore, SIS-treatment resulted in a large number of spindle shaped cells with more organized collagen matrix in the neo-PT tissue. These results clearly demonstrated the potential of SIS-treatment to increase synthesis of neo-PT tissue while preventing adhesion formation between the neo-PT and

infrapatellar fat pad. Indeed, these in-vivo animal studies reveal that SIS can be a successful biological agent to improve healing of both ligaments and tendons.

4 Future Directions

Many investigators have followed Professor Y.C. Fung's teachings, pioneering work, guiding principles, and ingenious theories and have made significant advances in characterizing the biomechanical and biochemical properties of ligaments and tendons as well as determining their contributions to joint kinematics and function. For the past three to four decades, fundamental studies on the tensile and viscoelastic properties of ligaments and tendons, together with biologic factors that change their properties have helped us to gain new insights into the importance of these connective tissues. A large body of research has furthered the basic understanding of their healing process after rupture, as well as identified appropriate replacement grafts. All this knowledge has found its way into clinical application for better patient outcomes.

For the future, we have identified exciting areas such as the new field of tissue engineering to modify the molecular and cellular responses to enhance the properties of the healing tissue. Even tissue regeneration has become a realm of possibility. In our research center, we have utilized in-vitro cyclic stretching and cell seeding of ECM bioscaffolds to induce matrix production which mimics ligaments and tendons. Following a concept of contact guidance, the aligned ECM bioscaffold collagen structure can cause cells to align which, in turn, produce better aligned neocollagen. Further, other novel processing techniques, including electrospinning, can be used to synthesize bioscaffolds with a highly aligned fiber morphology- an optimal structure to promote enhanced ligament and tendon healing (127, 128). In addition, ECM gels, made from digested ECM powder, have been recently fabricated (118). These scaffolds retain important bioactive agents and are injectable, making them advantageous for filling large wound sites. Ultimately, we will redirect our efforts to regenerate ligaments and tendons which do not heal easily, such as the ACL, regarded as the "holy grail" in sports medicine research.

In terms of ligament reconstruction by replacement grafts, it is time to move our focus towards in-vivo conditions to study the injury mechanism and to gain in-vivo kinematics data which will be reproduced on cadaveric knees utilizing the robotic/UFS testing system (Figure 11). 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 by data obtained in the experiments (a procedure advocated by Professor Fung), it will be possible to use the computational model to study complex external loading conditions. These models can also be used to obtain stress and strain distribution in the ACL as well as to develop a database for patients of different ages, genders, and sizes. All will help to develop better surgical procedures and optimize rehabilitation protocols. Furthermore, it is hoped that the technique can be extended to study other ligaments and tendons that are injured frequently, such as those in the shoulder.

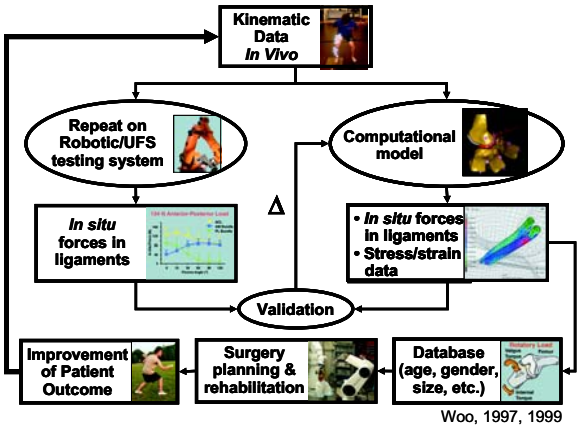

Figure 11: Flow chart showing the utilization of in-vivo kinematics data to drive experimental and computational methodologies leading to improved patient outcome.

Ligament and tendon research has reached an exciting time where the development of novel methods to improve the treatment of their injuries can become a reality. On the other hand, there is still

a long way to go to translate cellular responses to in-vivo situations and eventually to clinical application as the biology is so complex. 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, immunologists, and so on) will need to learn to work together in a seamless manner before one can regenerate ligaments and tendons to become near normal tissue. With that, we are optimistic that future patients will be able to resume and remain able to perform their normal daily activities as well as sports activities at a high level.

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