EFFECTS OF AGING ON THE LATERAL TRANSMISSION OF FORCE IN SKELETAL MUSCLE

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EFFECTS OF AGING ON THE LATERAL TRANSMISSION OF FORCE IN SKELETAL MUSCLE

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Aging of skeletal muscle, characterized by involuntary loss of muscle mass and strength during aging, has become one of extraordinary concerns for the increasing elderly population. The reduction of muscle strength is partially caused by decreased skeletal muscle cross-sectional area (CSA) with ageing; however, muscle force is lost to a greater extent than the loss of muscle mass, suggesting that other factors are involved. Changes in ATPase activity, metabolite levels, or myosin isoforms cannot fully explain the loss of specific force in aged muscles. These findings suggest that the force transmission pathway from myofibers to the tendon in aged muscles might be impaired, therefore, lead to the loss of skeletal muscle strength. The aim of this study is to characterize the effects of aging on force transmission in skeletal muscle and determine the underlying mechanisms.

In current study, firstly, we studied the mechanism of lateral transmission of force in skeletal muscle by a finite element model of single muscle fiber. We found that most of

the force generated in myofibers is transmitted near the end of the myofiber through shear stress to the surrounding ECM. Force transmitted to the end is affected by mechanical and geometrical properties of ECM. Secondly, effect of aging on lateral transmission was determined by isometric contractile tests on the extensor longus muscle with series of tenotomy and myotomy on young and old rats. Significant drop in force with myotomy was observed in old rats, indicating the impaired lateral transmission pathway with aging, which could be partly due to increased thickness of the ECM. Possible mechanisms of the age-related change in force transmission were further explored through combining the transmembrane proteins into finite element analysis. We found that force transmitted in the muscle is sensitive to the amount of proteins; more force could be transmitted in model with more transmembrane proteins. However, the force transmission is not sensitive to the stiffness of transmembrane proteins. The thesis was concluded by a discussion of study and possible future work.

BIOGRAPHICAL SKETCH

Chi Zhang earned his Bachelor of Science in Engineering degree in Mechanical Engineering from Tsinghua University, Beijing in 2009. He joined the doctoral program in Mechanical Engineering at Cornell University in 2009 and has since earned his Master of Science degree in Mechanical Engineering in 2012.

Chi has been the recipient of the Cornell Graduate Student Fellowship and Graduate Research Fellowship. In addition to his research experiences, Chi has been the teaching assistant of four courses provided by the department of Mechanical Engineering. He was also active in Chinese Students and Scholars Association in Cornell University.

Chi Zhang's dissertation was supervised by Dr. Yingxin Gao.

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Chapter 1

Introduction

The population over 65 years old in U.S. was 40.2 million in 2010, and is estimated to reach 88.5 million in 2050 (Administration on Aging, 2010). One of the major concerns for this elderly population is disease/disability related to aging of skeletal muscle. Aging of skeletal muscle causes sarcopenia, which is involuntary loss of muscle mass and strength. Approximately 45% of the older U.S. population is affected by sarcopenia (Janssen et al., 2004). The estimated annual healthcare cost directly related to aging of skeletal muscle was over $20 billion in 2010, and the number keeps rising with the increasing elderly population. Although the muscle change due to aging is inevitable, therapies and exercise that can delay or even reverse these effects are promising (Lynch, 2011). Studying the effects of aging on skeletal muscle and the underlying mechanisms requires knowledge of structure and physiology of skeletal muscles.

1.1 Structure and physiology of skeletal muscle

1.1.1 Structure of skeletal muscle

The skeletal muscle has a complex hierarchical structure, which is mainly composed of muscle fibers and the extracellular matrix (ECM) surrounding them. The

muscle fiber is a multinucleated single muscle cell (Figure 1.1). Every muscle fiber is composed of many myofibrils, with each myofibril composed of sarcomeres arranged in series along the length of the myofiber. Sarcomere is the basic functional unit of myofiber and responsible for striated appearance and actual force production. Within each sarcomere there is a network of numerous thick and thin filaments. The thick filaments are composed almost exclusively of the protein myosin, and the thin filaments (about half diameter of thick filaments) are mainly composed of the protein actin. The striated pattern created by the arrangement of thick and thin filaments is shown in Figure 1.2.

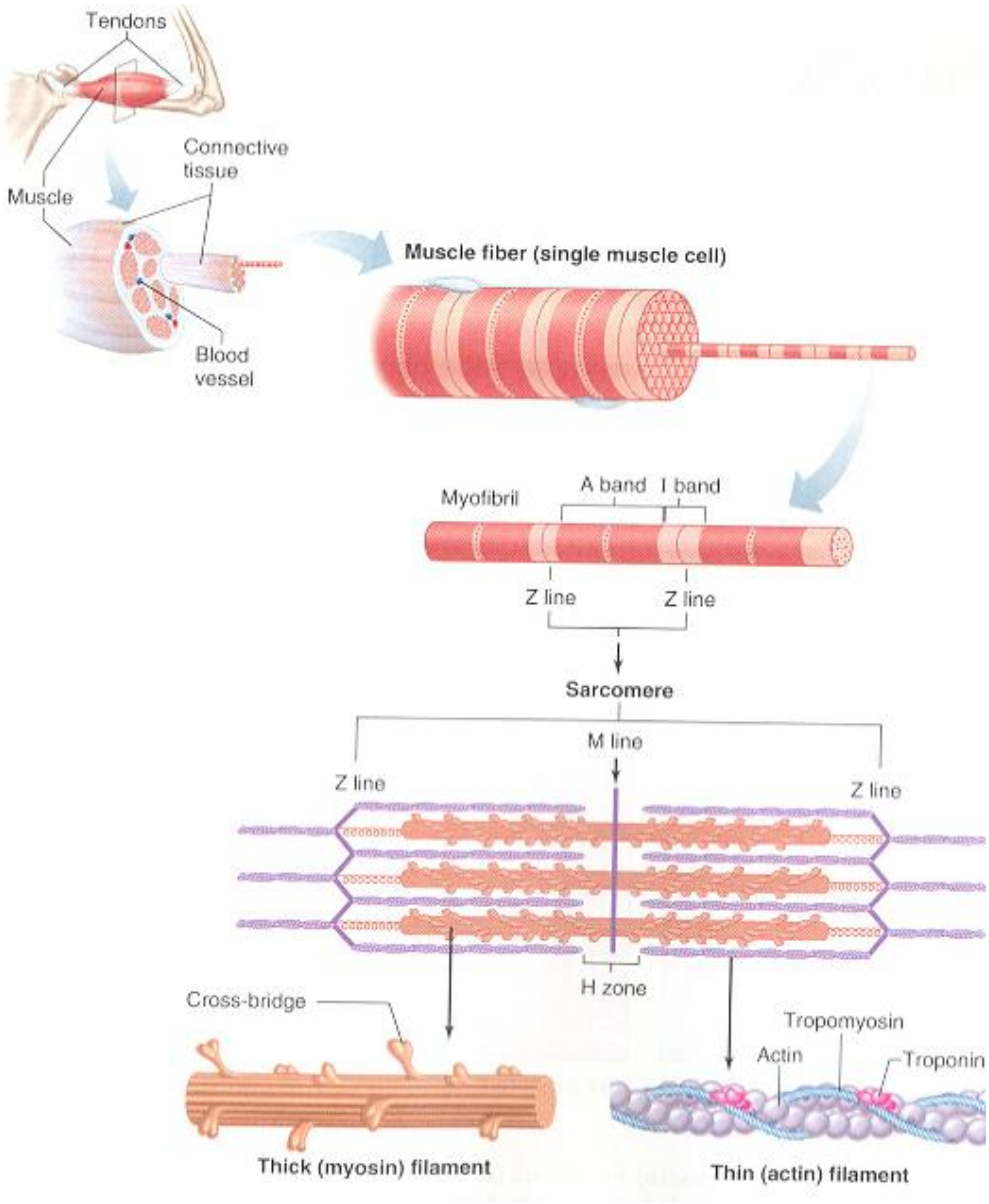
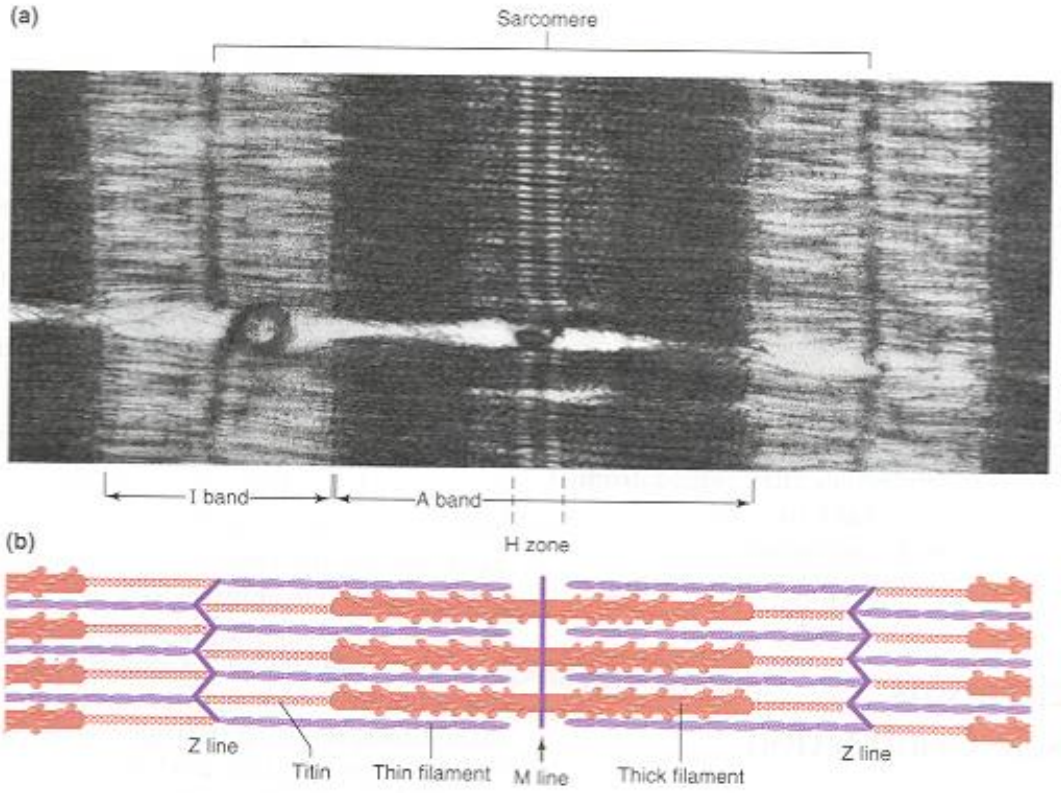


Figure 1.1 Schematic drawing of the structure organization of skeletal muscle. Adapted from Vander's Human Physiology, 11th Ed., McGraw-Hill, 2007.



FIigure 1.2 (a) High magnification image of a sarcomere within myofibrils showing the striated pattern. (b) Arrangements of thick and thin filaments in the sarcomere shown in (a). Adapted from Vander's Human Physiology, 11th Ed., McGraw-Hill, 2007.

1.1.2 Skeletal muscle contraction

During a muscle contraction, the contractive force in the sarcomere is generated by the overlapping thick and thin filaments moving past each other. This is the sliding filament theory for skeletal muscle contraction (Huxley, 1957). The underlying mechanism for the sliding filaments is: when skeletal muscle is stimulated, calcium ions are released and enter the cytoplasm. Under the effects of calcium and ATP, cross-bridges in the thick filaments bind to actins in the thin filaments, and move toward the

center of the sarcomere, which will generate contractive force. A schematic of this movement is shown in Figure 1.3. Based on the sliding filament and cross-bridge theory, forces could be generated during contractions is dependent on the overlapping section of thick and thin filaments and the sarcomere length. Experimental results of maximum isometric force developed at different sarcomere length perfectly match the sliding filament theory proposed. Active and passive force developed in sarcomere at different length is shown in Figure 1.4.

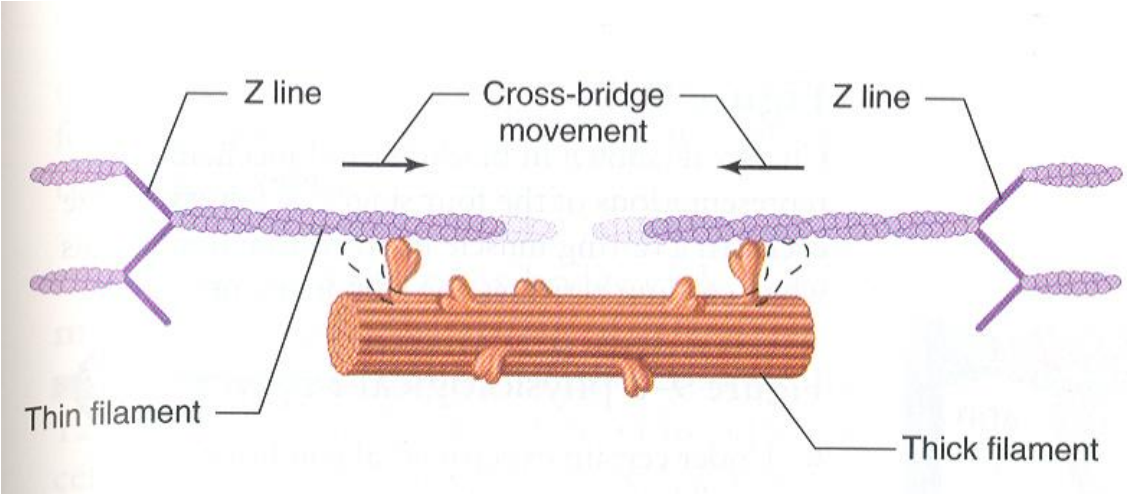


Figure 1.3 During muscle contraction, calcium ions are released and cross-bridges bind to actin in the thin filaments. The bonded cross-bridges propel the thin filaments toward the center of the sarcomere and generate force. Adapted from Vander's Human Physiology, 11th Ed., McGraw-Hill, 2007.

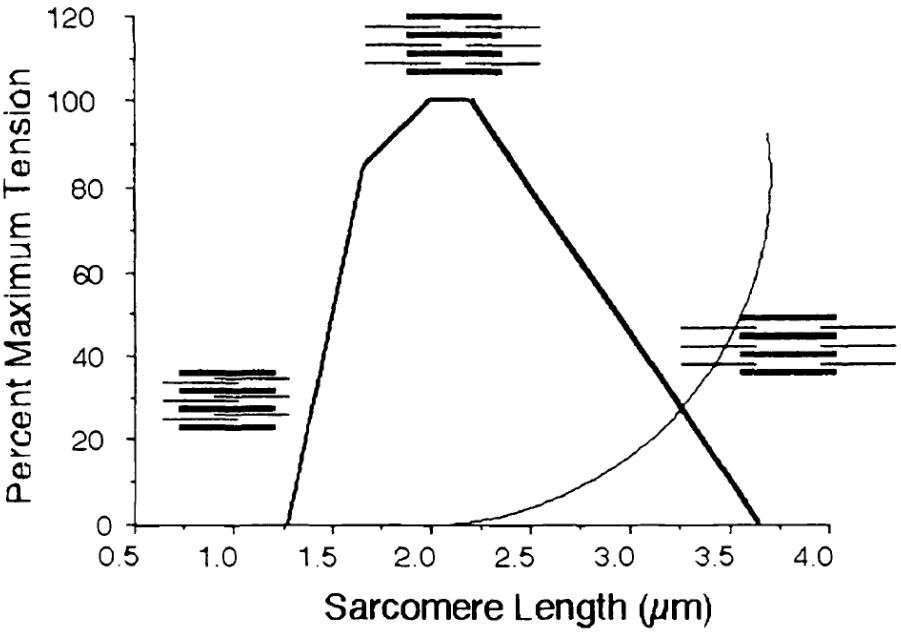


Figure 1.4 Sarcomere length-tension relationship demonstrating active force (thick lines) and passive force (thin curve) developed at different sarcomere lengths. The active force (thick line) in the figure is the maximum isometric force generated during contraction. Based on the sliding filament theory, maximum force can be generated is dependent on the amount of overlapped cross-bridges and actin sites. When the sarcomere is too short, overlapping of actins generates extensive interference, and the amount of cross-bridges could bind decrease to the left of the figure. When the sarcomere is stretched too much, only small overlapping happened between cross-bridges and actin sites, indicated by decreasing active force could be generated. Therefore, the active force-length curve of sarcomere is a sectional linear curve. The passive force-length curve (thin line) is a nonlinear curve based on the nonlinear material properties of sarcomere. Image adapted from literature (Lieber, 1999).

1.1.3 Extracellular matrix in skeletal muscle

The extracellular matrix (ECM) surrounding the myofibers is organized to three different structural levels: the endomysium that surrounds every single myofiber; the perimysium that binds the muscle fascicles; and the epimysium that encompasses the entire skeletal muscle (Figure 1.5). ECM provides structural support for integrity and

ensures passive elastic response of the whole muscle. More importantly, interactions between the ECM and myofibers determine the mechanical behaviors of skeletal muscles (Kjaer, 2004). The unique structural, biochemical and biophysical properties of the ECM make it an important structure in passing mechanical signals into the cell to produce a signaling cascade through molecules that connect the ECM to muscle cells (Lieber, 2002). Changes in the ECM will cause altered mechanical environments around the cell through this interactions, and therefore initiate the mechanotransduction process in muscle (Kjaer, 2004; Purslow, 2002), which affects the muscle adaptation to aging, injury, disease, and the outcomes of corresponding treatments.

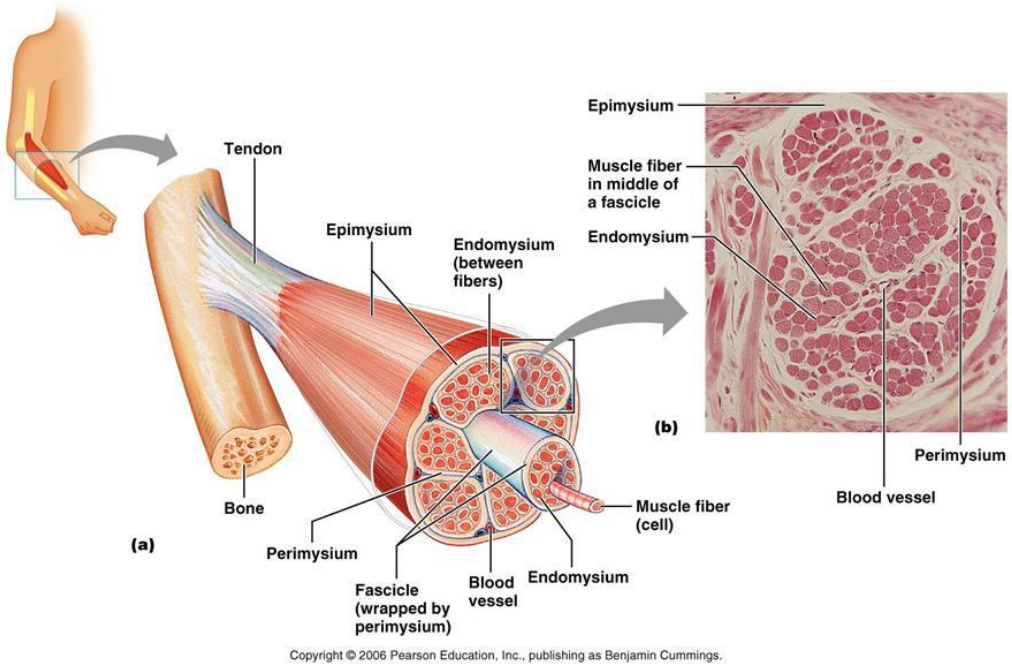


Figure 1.5 Demonstration of different levels of extracellular matrix in skeletal muscle: endomysium, perimysium, and epimysium. Image adapted from Essentials of Human Anatomy and Physiology, Pearson Education, Inc., 2006.

1.2 Force transmission in skeletal muscle

In order to generate body movements, force generated by the activation of contractile molecules within the muscle needs to be finally transmitted to tendons that connect to the bones. The contractile force in sarcomere can be transmitted both in series and in parallel to adjacent sarcomeres and then across cellular membrane of muscle fiber (Huijing, 1999). After reaching the membrane, that force can be transmitted longitudinally through the myotendinous junction, as well as laterally between muscle fibers and the extracellular matrix network (Street, 1983).

1.2.1 Longitudinal transmission of force

The morphological structure of the connecting region between the muscle and the tendon is identified as the myotendinous junction (MTJ). At the location of MTJ, the actin filaments extending from the last A-band bind to sarcolemma through transmembrane proteins (Tidball, 1991a, 1991b; Trotter, 2002). Two separate sets of transmembrane proteins have been identified in the MTJ: dystrophin and dystrophin-associated glycoprotein complex (DGC) and the $\alpha 7\beta 1$ integrin system. The DGC distributes both in MTJ and along the lateral membrane of myofibers, and supports the strength of muscle cell membranes. The integrin system is very important at MTJs serving as both mechanical linkage and components of signal transmission system (Giancotti & Ruoslahti, 1999).

The extensive folding of sarcolemma in MTJ provides the structural support for force transmission at the end (Huijing, 1999). As myofibers approach their tendinous origin or insertion, their diameters decrease significantly (Trotter, 1993). Sarcolemma folds extensively along the fiber direction. The folding significantly increases the surface area available for force transmission, and therefore, decreases the magnitude of the stresses at the site (Tidball, 1991a; Trotter, 1993).

1.2.2 Lateral transmission of force

For muscles of most species, muscle fiber commonly ends within the fascicles without reaching the myotendinous junction (Gaunt and Gans, 1992; Trotter, 1993; Trotter, 2002; Trotter and Purslow, 1992), as shown in Figure 1.6, suggesting that the force generated in one muscle fiber must be transmitted laterally via the surrounded endomysium to adjacent fibers or ECM, and finally to the tendon (Huijing, 1999). Such force transmission was defined as lateral transmission of force (Huijing, 1999; Monti et al., 1999). The existence of lateral transmission of force has been demonstrated experimentally on structural levels of single muscle fibers, fascicles, and even different muscles (Balice-Gordon and Thompson, 1988; Huijing, 1998; Street and Ramsey, 1965; Street, 1983).

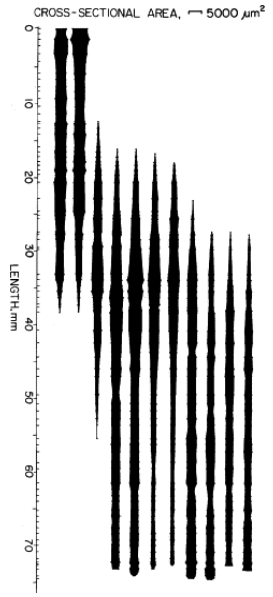


Figure 1.6 Muscle fibers end within the fascicles without reaching the myotendinous junction. Image adapted from literature (Ounjian et al., 1991).

Two chains of proteins discontinuously distributed along the interface between the myofiber and the surrounding ECM form the pathways for lateral transmission of force across fiber membranes (Lieber, 2002). As shown in Figure 1.7, In the first chain, called dystrophin–glycoprotein complex (DGC), the actin binds to dystrophin, and then to dystroglycan in the sarcolemma, and then finally to collagens in the extracellular matrix. DGC can transmit the pulling force from actin to the endomysium, as well as support muscle fiber strength, increase sarcolemmal deformability, and stabilize the microstructure within myofiber to prevent muscle fiber injury. In a second chain of proteins, actin binds to talin, which binds vinculin, and then to integrin, and finally to collagen in the ECM (Tidball, 1991a). The chain of talin-vinculin-integrin is not only the pathway of force transmission but also a crucial component for focal adhesion and the

mechanotransduction process in skeletal muscle (Danowski et al., 1992; Tidball, 1991a; Balaban et al., 2001; Hu et al., 2007).

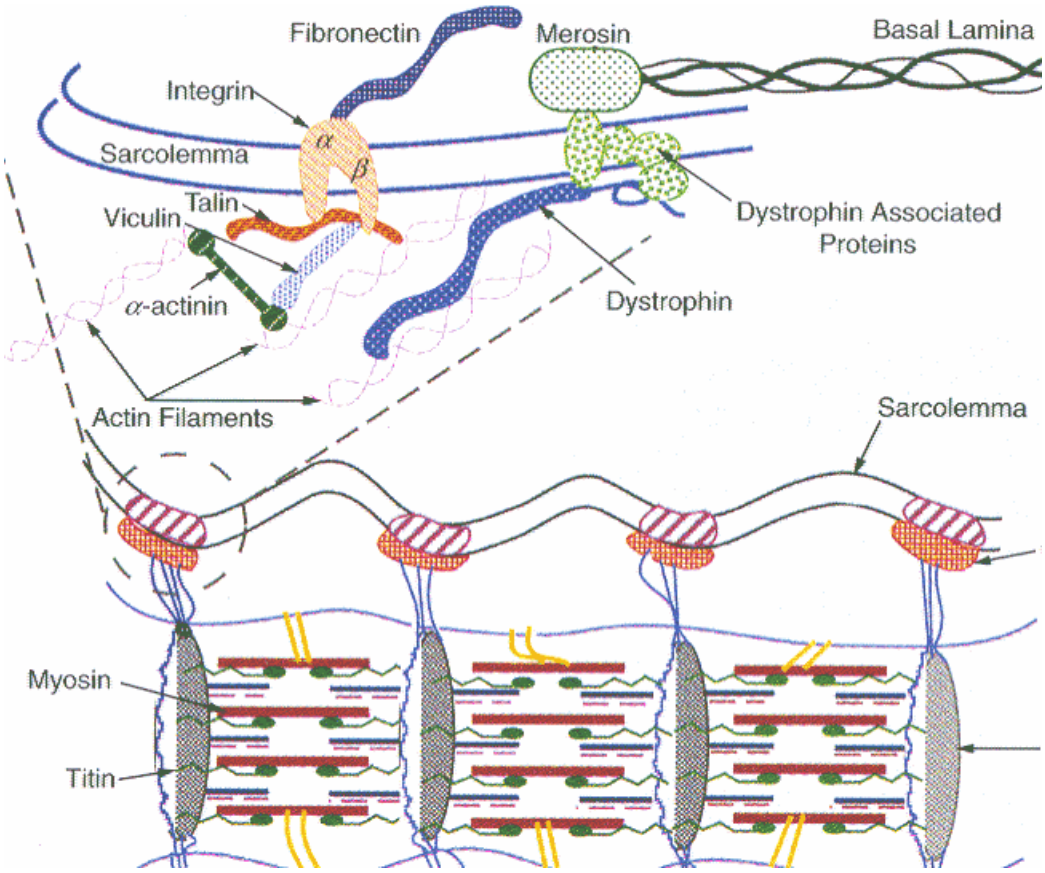


Figure 1.7 Generalized diagrammatic representation of the protein pathways taking part in force transmission from myofiber to the extracellular matrix. Image adapted from Skeletal Muscle Structure, Function and Plasticity, Lieber, Williams and Wilkins, 2002.

1.3 Effects of aging on skeletal muscle

The strength of skeletal muscles is reduced due to aging (Siegal, 1996; Rogers & Evans, 1993). This reduction is partially caused by decreasing skeletal muscle cross-sectional area (CSA) with aging (Frontera et al., 2000; Lexell et al., 1988; Kent-Braun et

al., 2000). However, muscle force is lost to a greater extent than the loss of muscle mass, suggesting that other factors are involved (Nair, 2005). Changes in ATPase activity, metabolite levels, or myosin isoforms have been proposed to be the possible mechanisms; however, none of them can fully explain the loss of force in aged muscles (Philips et al., 1993; Lowe et al., 2001; Lowe et al., 2002). Studies on rodent models showed that although the specific force (force per area) of the whole muscles decreases with aging (~13% - 20%), there is no significant deficit in specific force of single skinned muscle fibers between the young and old groups (Eddinger et al., 1986; Brooks and Faulkner, 1988, 1994; Philips et al., 1991). The deficit in specific force of whole muscles cannot be explained by unimpaired intrinsic force-generating capacity of cross bridges in aged muscles, suggesting that the force transmission from muscle fibers to the tendon in aged muscles could be impaired, leading to the loss of skeletal muscle strength.

The stiffness and the thickness of ECM in skeletal muscles increase with aging (Nishimura et al., 1997; Kjaer, 2004; Gao et al., 2008), the force transmission, especially the lateral transmission of force, could be affected due to these changes. Changes of amount and type of transmembrane proteins related to force transmission during aging could also affect the force transmission. However, results of the number and type of proteins that are affected by aging from different studies are controversial and unclear, and these changes are also stated to be depended on the type of skeletal muscle (Ramaswamy et al., 2011; Rice et al., 2006).

1.4 Mathematical models of skeletal muscle

Skeletal muscle has a complicated microstructure, and transmission of force involves very complicated mechanical interactions between muscle fiber and the ECM. Aging is associated with changes in geometrical, structural and mechanical properties of ECM, muscle fibers and the molecules in between. Experimental results can only show the overall effects of all changes in structural and mechanical properties, and therefore it is difficult to experimentally determine the mechanisms of age related changing on strength of skeletal muscles.

Mathematical models have the advantage in manipulating variables which are otherwise difficult to do experimentally. The most commonly used mathematical models to describe forces generated in myofibers are the Hill Model and the Huxley Model. The Hill Model is a phenomenological model in which microstructures of muscles are not incorporated; therefore, it is not able to provide structural mechanisms of muscle contractions (Herzog, 2000). In contrast, the Huxley's model is a model including structures that affect the force generation, in which force generated in myofibers is described as a function of attachments of cross-bridges (Huxley, 1957). Zahalak further developed the Huxley Model by incorporating chemical changes during muscle contractions and introducing a distribution-moment (DM) method to save computing expense (Zahalak, 1981; Zahalak and Ma, 1990).

Most previous mathematical models considered skeletal muscles as one tissue without separating the structures of myofibers and the ECM, therefore, no information

about force transmission between them can be determined from those models (Blemker et al., 2005; Chen and Zeltzer, 1992; Johansson et al., 2000; Kojic et al., 1998; F.C.T. van der Helm, 1994). Yucesoy et al. (2002) developed a FE model, in which two single layers of mesh, the muscle fiber layer and the ECM layer, were linked elastically. However, the geometry of that model could not represent the physiological structure of long muscles, in which the muscle fibers terminate intrafascicularly.

1.5 Specific aims

The overall goal of this thesis was to determine the effects of aging on the lateral transmission of force in the skeletal muscle, and to investigate the possible underlying mechanisms. Both experimental and computational studies are involved in this study to understand how age related changes in structures and properties of skeletal muscles affect the force transmission within skeletal muscle.

1.5.1 Mathematical model of force transmission between myofiber and endomysium

The purpose of this part was to determine the mechanisms of lateral transmission of force between myofibers and the extracellular matrix (ECM). A finite element (FE) model of single muscle fiber composed of a myofiber and surrounding endomysium was developed, and force transmitted to the end of the single muscle fiber is calculated. Efficiency of the lateral transmission and the stress distribution within the

myofiber and on the myofiber-endomysium interface were analyzed to determine how the force generated in myofiber was transmitted through the interactions between myofiber and the endomysium.

1.5.2 Effects of aging on the lateral transmission of force in skeletal muscle

The aim of this part was to determine the effect of aging on the lateral transmission of force in skeletal muscles. Forces transmitted to the end of the extensor digitorum longus muscle (EDL) of rats during isometric contractions were measured *in vitro* and compared between young and old groups. Series of tenotomy and myotomy were performed on the muscle to measure the force transmitted longitudinally and laterally. The FE model of the single muscle fiber was used to explore possible mechanism for the force transmission change during aging.

1.5.3 The role of transmembrane proteins on the force transmission in muscle

To further discuss the underlying mechanism for the age related change of lateral transmission, effects of changes in interfacial proteins between myofiber and ECM on the lateral transmission of force are studied. A model consisted of myofiber, ECM, and the transmembrane proteins between them has been proposed, and parametric study on the amount and mechanical properties of the lateral proteins are also conducted.

1.6 Chapter overviews

To accomplish the specific aims, a detailed understanding of the structure and physiology of the skeletal muscle is required. The aging of skeletal muscle, the mechanism of force generation, and pathways of force transmission are introduced as the background of this thesis in the current Chapter 1.

Chapter 2 describes a 2D finite element model of single muscle fiber. The model is composed of a single myofiber and the surrounded endomysium. Theory of nonlinear elasticity and a physiological based mathematical model of active force generation are combined in the model. Effects of mechanical properties of the endomysium and the tapered ends of myofiber on lateral transmission of force in skeletal muscle are studied to better understand the mechanism of lateral transmission.

The purpose of Chapter 3 is to determine the effects of aging on the lateral transmission of force in rat skeletal muscles. In this chapter, proportions of force transmitted laterally and longitudinally to the end of the EDL muscle are measured and compared between young and old rats. A mathematic model is involved to discuss possible mechanism for the experimental observation.

In Chapter 4, a finite element model is developed to study effects of changes in muscle fiber membrane proteins on the lateral transmission of force. The membrane proteins are modeled as lateral linkages connecting the myofiber and ECM, with nonlinear elastic properties determined based on previous studies. Effects of amount change and stiffness change of lateral linkages on the force transmitted are studied.

Finally, Chapter 5 summarizes the most important conclusion of this thesis. Directions for the future research are proposed.

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Chapter 2

Finite Element Analysis of Mechanics of Lateral Transmission of Force in Single Muscle Fiber[1]

2.1 Introduction

Skeletal muscles have a complex hierarchical structure which is mainly composed of myofibers and the extracellular matrix (ECM) surrounding them. There are three different structural levels of the ECM: endomysium that surrounds every single myofiber; perimysium that binds the muscle fascicles; and epimysium that encompasses the entire skeletal muscle. ECM not only provides structural support to ensure integrity of the whole muscle, but more importantly, interactions between the ECM and myofibers determine the mechanical behaviors of skeletal muscles**.** The unique structural, biochemical and biophysical properties of the ECM make it an important structure in passing mechanical signals into the cell to produce a signaling cascade through molecules that connect the ECM to muscle cells (Lieber, 2002). Changes in the ECM will cause altered mechanical environments around the cell through this

1. Zhang, C., Gao, Y., 2012. Finite element analysis of mechanics of lateral transmission of force in single muscle fiber. Journal of Biomechanics 45, 2001-2006.

interactions, and therefore initiate the mechanotransduction process in muscle (Kjaer, 2004; Purslow, 2002), which affects the muscle adaptation to aging, injury, disease, and the outcomes of corresponding treatments.

The mechanical interactions between muscle cell and the ECM are mainly occurring through the force transmission between them. It has been shown that for many muscles, across species, muscle fiber commonly ends within the fascicles without reaching the myotendinous junction (Gaunt and Gans, 1992; Trotter, 1993; Trotter, 2002; Trotter and Purslow, 1992). This suggests that the force generated in these muscle fibers must be transmitted laterally via the endomysium. Such pathway of force transmission was defined as lateral transmission of force (Huijing, 1999; Monti et al., 1999). Although the existence and necessity of lateral transmission of force has been demonstrated experimentally between single muscle fibers and fascicles, and even different muscles (Balice-Gordon and Thompson, 1988; Huijing, 1998; Street and Ramsey, 1965; Street, 1983), the mechanical mechanism of this transmission is not well understood.

Skeletal muscle has a complicated microstructure, and therefore it is difficult to experimentally determine the mechanisms of lateral transmission of force. Mathematical models have the advantages in manipulating variables which is otherwise difficult to do experimentally. The most commonly used mathematical models to describe forces generated in myofibers are the Hill Model and the Huxley Model. The Hill Model is a phenomenological model in which microstructures of muscles are not incorporated; therefore, it is not able to provide structural mechanisms

of muscle contractions (Herzog, 2000). In contrast, the Huxley's model is a model including structures that affect the force generation, in which force generated in myofibers is described as a function of attachments of cross-bridges (Huxley, 1957). Zahalak further developed the Huxley Model by incorporating chemical changes during muscle contractions and introducing a distribution-moment (DM) method to save computing expense (Zahalak, 1981; Zahalak and Ma, 1990). However, the Zahalak's model is a model of sarcomeres based on the cross-bridge theory, and it is only focused on myofiber without surrounding endomysium. Therefore it cannot be directly used to study the interactions between myofibers and the ECM.

Most previous mathematical models considered skeletal muscles as one tissue without separating the structures of myofibers and the ECM, therefore, no information about force transmission between them can be determined from those models (Blemker et al., 2005; Chen and Zeltzer, 1992; Johansson et al., 2000; Kojic et al., 1998; F.C.T. van der Helm, 1994). Yucesoy et al. (2002, 2003) developed a FE model, in which two single layers of mesh, the muscle fiber layer and the ECM layer, were linked elastically. However, the geometry of that model could not represent the physiological structure of long muscles, in which the muscle fibers terminate intrafascicularly.

The objective of this study was to determine the mechanisms of lateral transmission of force between myofibers and the ECM. We developed a 2D FE model of single muscle fiber with two separate tissues, myofiber and endomysium. The Zahalak's model was incorporated in the FE model to describe the active force

generated in the myofiber during contractions. Stress distributions along the myofiber and the interface between myofiber and endomysium were analyzed. Parametric studies were performed to determined effects of mechanical properties of the endomysium and tapered end of myofiber on the lateral transmission of force between myofiber and the endomysium.

2.2 Methods and Models

A fiber-reinforced composite is a material that is composed of two different components with discontinuous and strong fibers being embedded in relatively compliant matrix. Muscle is functionally a fiber-reinforced composite consisting of an ECM with reinforcement by myofibers (Huijing, 1999). The basic structural unit of the muscle is composed of one myofiber and the surrounding endomysium. In this study, the force transmission between the myofiber and the endomysium within such one structural unit, i.e., one single muscle fiber was studied.

2.2.1 Model description

The geometry of a simplified single muscle fiber, or one structural unit, was shown in Figure 2.1, in which the myofiber is surrounded by the endomysium. The interface between the myofiber and the endomysium was modeled as perfectly bonded. For 2D problems, both myofiber and endomysium were modeled as rectangular shape.

Both ends of the single fiber model were fixed to simulate isometric contraction, by which no injury will be induced. To reduce computational time, only the upper right quarter of the model, which is in the dashed line in Figure 2.1, was calculated due to the geometric symmetry of this model. Symmetric boundary conditions were applied on symmetry axes.

Physiological structures of the tapered end of single muscle fiber have been well observed in previous studies (Barrett, 1962; Eldred et al., 1993; Gaunt and Gans, 1990; Trotter, 1990). For the purpose of geometric simplification, a trapezoid shape and a constant angle of fiber end were chosen for tapered ends. Based on the previous observation on the ratio between cross-section area and the taper length (Eldred et al., 1993), effects of tapered end on force transmission were determined by changing the rectangular ends to the ends with 5^{o} and 15^{o} tapered angles (Figure 2.2). Although the angles may not reflect the physiological geometry, the purpose of this study is to determine the sensitivity of lateral transmission of force between myofiber and the endomysium to the taper angle.

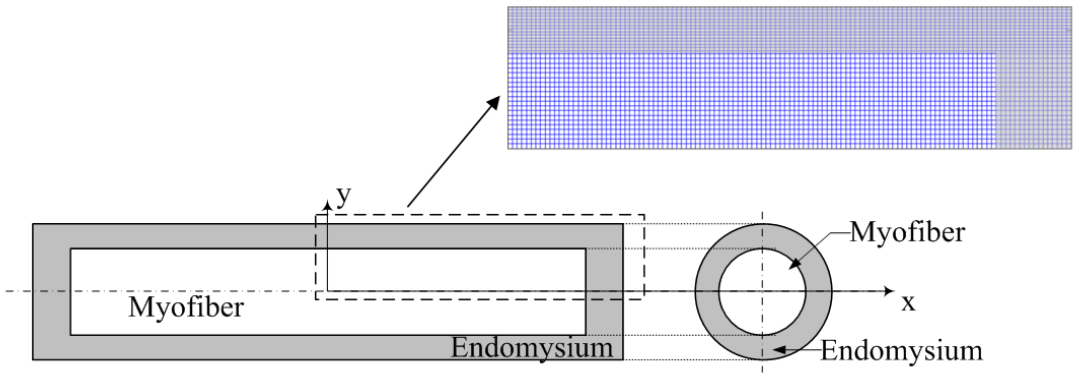

Figure 2.1 Schematic diagram of 2D single muscle fiber model. The single muscle fiber is composed of myofiber and the surrounding endomysium.

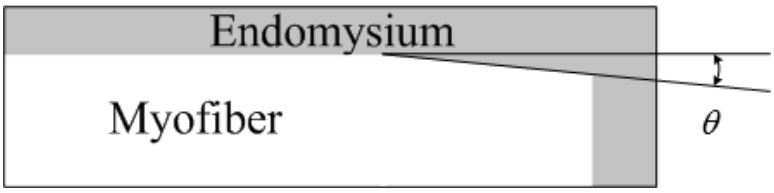

Figure 2.2 End of myofiber with $\theta = 5^o$ *and* $\theta = 15^o$ *tapered angels*

A 2 second, 100Hz stimulation signal was applied to myofibers to induce a tetanic contraction. Total force transmitted was calculated as the reaction force at the right end of single muscle fiber, and stress distributions of the interfacial shear stress, τ, and the tensile stress in the myofiber, σ were calculated (Figure 2.3). The role of ECM on force transmission was then determined by changing the stiffness of the endomysium.

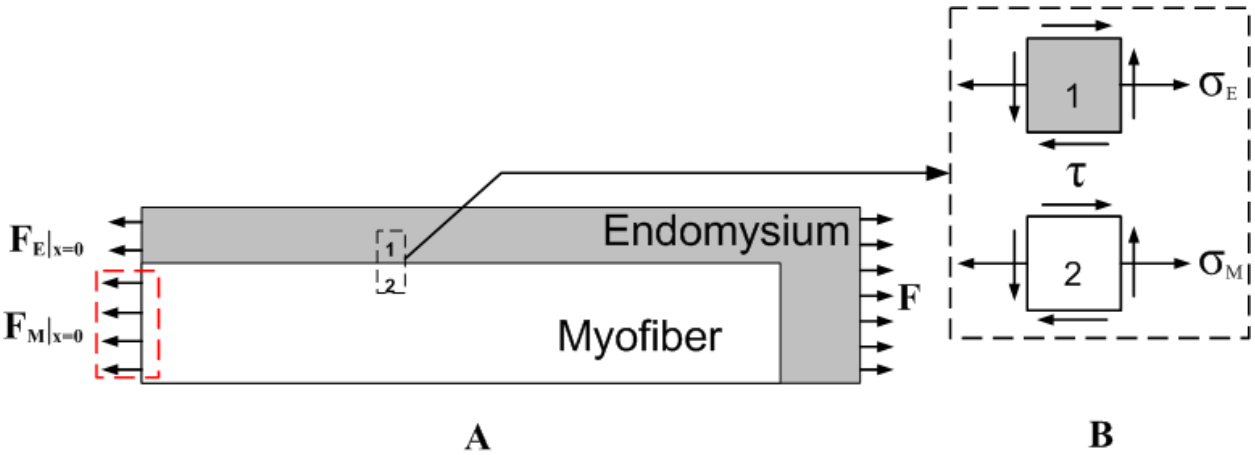

Figure 2.3 A. Free body diagram of the single muscle cell, or the structural unit, during muscle contraction. F is the total force transmitted to the end of the single fiber and is calculated as reaction force at the right end of the single muscle fiber. F_M represents the tensile load in the myofiber, and $F_M|_{x=0}$ represents the force in myofiber at $x=0$. F_E represents the tensile load in the endomysium, and $F_M|_{x=0}$ represents the force in the endomysium at $x=0$. Equilibrium in force requires that $F_M+F_E=F$ at any transversal cross section along x. The force in myofiber, $F_M=F_a+F_p$, in which F_a and F_p are the active force and the passive force in the myofiber, respectively. B. Stress state of elements in the endomysium and the myofiber.

2.2.2 Modeling the active stress of myofibers

Active stress in myofiber during contraction is calculated using Zahalak's model (Zahalak and Ma, 1990) in which distribution of bonded cross-bridge density $n(x,t)$ is determined by net attachment and detachment rates of bonded cross-bridge, $f(x)$ and $g(x)$, and the relative shortening velocity $v(t)$ between actin and myosin:

$$\frac{\partial n}{\partial t}-v(t)\frac{\partial n}{\partial x}=r\left([Ca]\right)f(x)(1-n)-g(x)n \qquad (2.1)$$

where $r([Ca])$ is the function of sarcoplasmic free calcium concentration defined as:

$$r([Ca]) = \frac{k_1^{\,2}[Ca]^2}{k_1^{\,2}[Ca]^2 + k_1 k_{-1}[Ca] + k_{-1}^{\,2}} \tag{2.2}$$

In Eq. (2.2), $[Ca]$ is the concentration of free Ca^{2+} ions in the sarcoplasm, and k_1 and k_{-1} are binding and release rates of calcium which can be determined by stimulation signals and initial calcium concentrations. The distribution-moment method developed by Zahalak (1981) is then used to calculate force generation. A more detailed method for calculating the active stress in the myofibers, please refer to the Appendix or previous study by Zahalak (1981).

2.2.3 FEM implementation of the model

To reduce the complexity of muscle contraction, the contraction process was modeled as small discretized time steps in this study. Within each step, the muscle contraction was modeled as a quasi-static problem, and viscoelastic behaviors of the model were neglected. Principle of virtual work was applied to solve for nodal displacements at each time step. The Total Lagrange (TL) formulation, in which all quantities are measured with respect to the original configuration at t_0, is used to describe the deformation. The principle of virtual work at the time $t + \Delta t$ is described as:

$$\int_{{}^0V} {}^{t+\Delta t}_{\ 0}S_{ij}\,\delta\,{}^{t+\Delta t}_{\ 0}E_{ij}\,d\,{}^0V = {}^{t+\Delta t}\Re \tag{2.3}$$

where:

$$ {}^{t+\Delta t}_{0}S_{ij} = {}^{t}_{0}S_{ij} + {}_{0}S_{ij} \tag{2.4} $$

$$ {}^{t+\Delta t}_{0}E_{ij} = {}^{t}_{0}E_{ij} + {}_{0}E_{ij} \tag{2.5} $$

in which ${}_{0}\mathbf{S}$ and ${}_{0}\mathbf{E}$ are the incremental growth of the stress and strain tensors from time t to time $t+\Delta t$. ${}^{t}_{0}\mathbf{S}$ and ${}^{t}_{0}\mathbf{E}$ are the second PK stress tensor and the Green strain tensor at the end of time t related to the original configuration at the time t_0.

The stress tensor $\mathbf{S}$ is calculated as a summation of active stress induced by contraction and passive stress due to contraction induced deformation:

$$ S_{ij} = S_{ij}^{\ pass} + S_{ij}^{\ act} \tag{2.6} $$

The active stress component $S_{ij}^{\ act}$ in myofiber is calculated by Zahalak's model, and is zero in the endomysium. Both the ECM and the passive behavior of myofiber were modeled as nearly incompressible Mooney-Rivlin material.

Nonlinear terms in Eqn. 2.3 were then linearized, and the Newton-Raphson method was applied to solve the nodal displacement within this step. The final matrix form of the problem can be written as:

$$ \left\{ \sum_e \int_{{}^0V_e} ({}^{t}_{0}\mathbf{B}_L^{\mathbf{T}}\, {}^{t}_{0}\underline{\underline{\mathbf{C}}}\, {}^{t}_{0}\mathbf{B}_L + {}^{t}_{0}\mathbf{B}_{NL}^{\mathbf{T}}({}^{t}_{0}\mathbf{S}^{pass} + {}^{t+\Delta t}_{0}\mathbf{S}^{act})\, {}^{t}_{0}\mathbf{B}_{NL})\, d\,{}^{0}V_e \right\} u = \sum_e \int_{{}^0V_e} {}^{t}_{0}\mathbf{B}_L^{\mathbf{T}}({}^{t}_{0}\hat{\mathbf{S}}^{pass} + {}^{t+\Delta t}_{0}\hat{\mathbf{S}}^{act})\, d\,{}^{0}V_e \tag{2.7} $$

where the incremental nodal displacement vector u is the quantity needed to be solved in each iteration, and $\mathbf{B}_L$, $\mathbf{B}_{NL}$ are matrices of the linear and nonlinear part of strain from nodal displacements, respectively (Bathe, 1996). Nodal displacements

calculated at the end of the current iteration were then used to calculate new ${}_0^t\mathbf{B}_L^{\mathrm{T}}$, ${}_0^t\mathbf{B}_{NL}^{\mathrm{T}}$, and ${}_0^t\underline{\underline{\mathbf{C}}}$ matrices for the next iteration step. The final displacement at time $t+\Delta t$ was determined only when both displacement and out-of-balance load convergence criteria were satisfied in the model. The entire model was implemented in MATLAB R2011a (MathWorks, Inc. Natick, MA).

2.2.4 Parameters

The values for each parameter we used for the myofiber and the endomysium were listed in Table 2.1.

Table 2.1. Values for each parameters used in the model

Model Parameters of Active Force Model[a]										
f_1	g_1	g_2	f_0	f_1'	τ_1	τ_2	k_m	b	ρ	τ
30	8	170	20	20	0.005	0.001	0.006	0.7	2	0.25
[s^{-1}]	[s^{-1}]	[s^{-1}]	[s^{-1}]	[s^{-1}]	[s]	[s]	[-]	[-]	[-]	[s]

[a] Taken from Zahalak and Ma (1990)

Model Parameters of Passive Properties of Myofiber and Endomysium Model			
	C_1[kPa]	C_2[kPa]	K[kPa]
Endomysium (control)[b]	0.3	0.15	10
Endomysium (high stiffness)[c]	0.6	0.3	20
Myofiber[d]	3	1.5	100

[b] Determined from Sharafi et. al. (2011)
[c] Determined from Gao (2008) and Sharafi (2011)
[d] Determined from Sharafi et. al. (2011)

2.3 Results

2.3.1 Forces transmitted to the end of the model

Total force transmitted to the end of the model as a function of time and ECM stiffness is shown in Figure 2.4. The total force transmitted is normalized by maximum active force generated in the myofibers during contraction. The model with low and high ECM stiffness transmitted 52.9% and 87.1% of the total force generated in myofiber, respectively. Therefore, increasing the ECM stiffness without changing myofiber properties resulted in larger force transmitted to the end of muscle fiber.

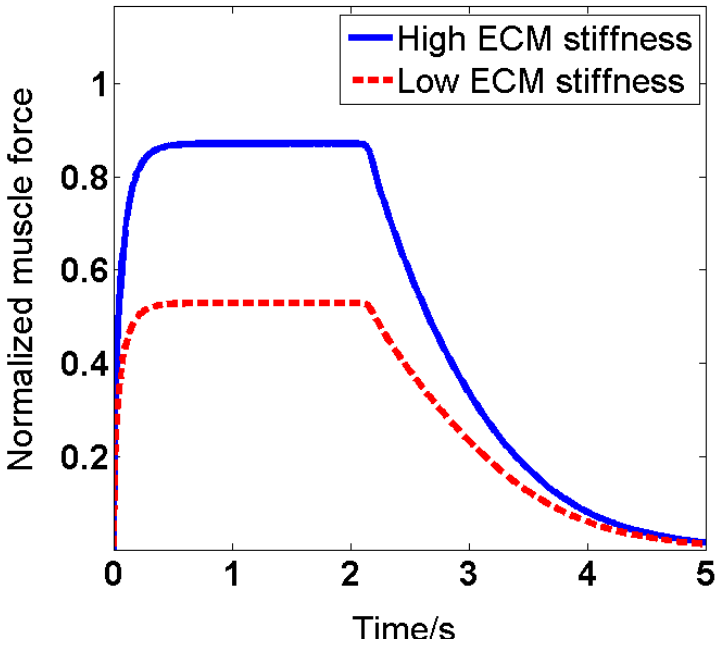

Figure 2.4 Force transmitted to the end of fiber, i.e., F, with different ECM stiffness. In this figure, all forces were normalized by the active force generated in the myofiber, i.e., F_a as defined in Figure 2.3.

2.3.2 Stress distribution along myofiber-ECM interface

Tensile stress and shear stress distributions along the myofiber of the model were shown in Figure 2.5. In the rest of the paper, unless otherwise mentioned, the x coordinates are normalized by the myofiber length, L, and the y coordinates are normalized by the average tensile stress on the middle cross-section of myofiber. The tensile stress is maximal in the middle of the fiber, and decreases to minimum at the end of myofiber. The shear stress has a minimum value in the middle of myofiber, and reaches a maximum value at the end.

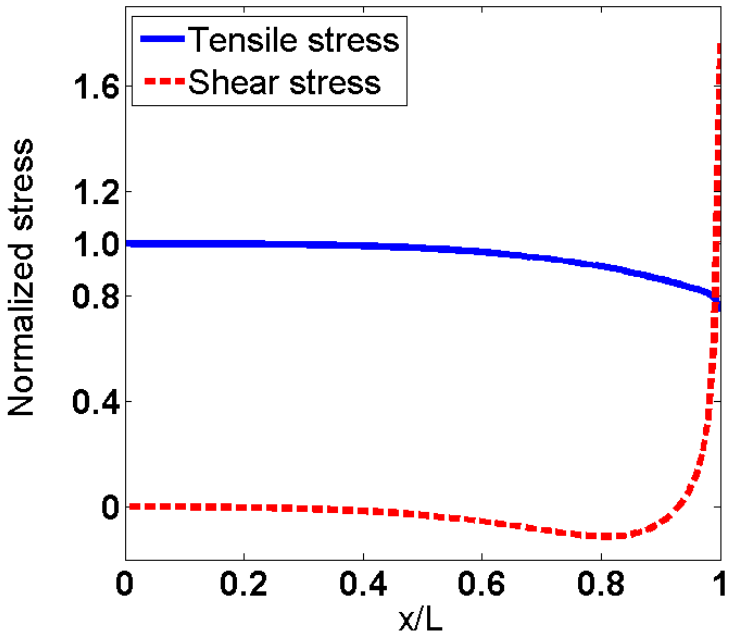

Figure 2.5 Tensile stress (σ) and interfacial shear stress (τ) along the longitudinal direction of myofiber

2.3.3 Stress distributions in myofibers with tapered ends

Figure 2.6 and Figure 2.7 show the effect of tapered angle on distributions of tensile stress and interfacial shear stress. Both tensile stress and the maximum shear stress at the end of myofibers decrease as the tapered angle increases. Forces that are transmitted through tensile stress in myofibers with different tapered angles are compared in Figure 2.8. The y coordinates are normalized to force in the middle of myofiber without tapered end. Figure 2.8 shows that force transmitted along the myofiber decreases with increased tapered angle. The myofiber without tapered end has the highest force distribution along the whole myofiber. The tensile force at the end of the myofiber in the 5^{o} and 15^{o} tapered end model were 79.06% and 40.96% of that in the one without tapering, respectively.

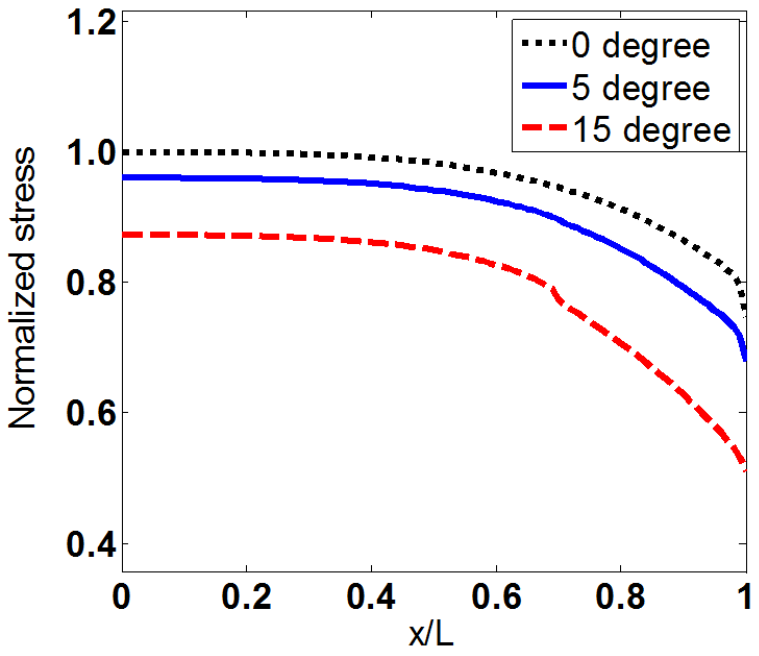

Figure 2.6 Tensile stress distributions along myofibers with different tapered angles

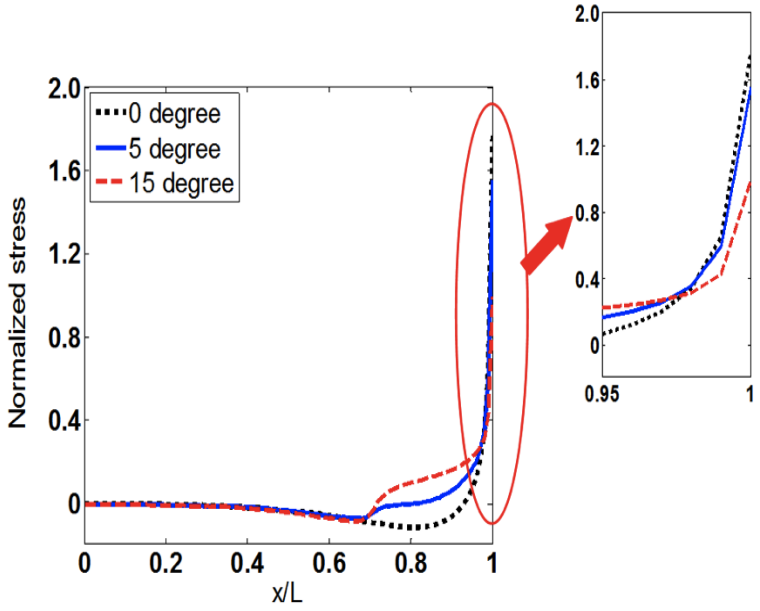

Figure 2.7 Interfacial shear stress distributions along the myofiber with different tapered angles

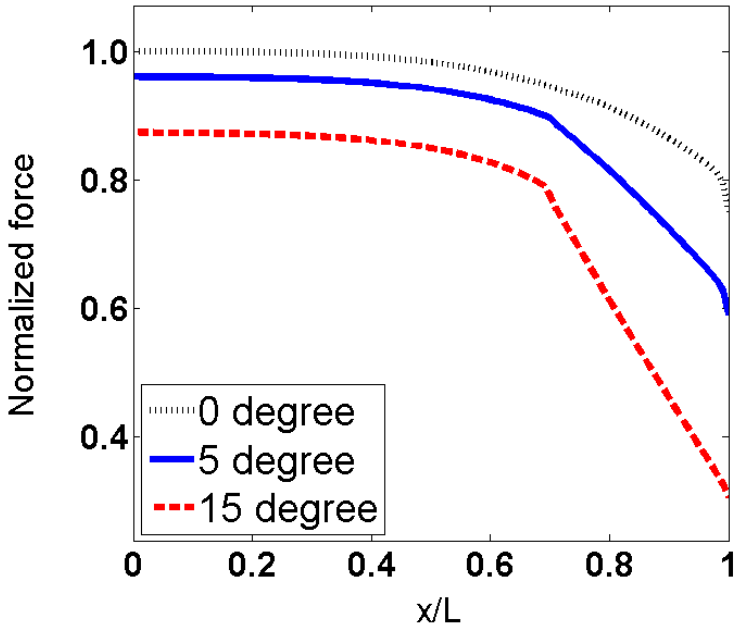

Figure 2.8 Normalized tensile force distribution along the longitudinal direction of myofiber with different tapered angles

2.3.4 Von Mises stress distributions along myofiber-ECM interfaces

Von Mises stress distributions along the myofiber-ECM interface in models with different tapered angles are shown in Figure 2.9. Von Mises stresses are constant near the middle of myofiber, and reach maximum values at the ends of the myofibers. The myofiber without tapering end has the largest von Mises stress at the end, and the one with a 15^{o} tapered angle has the lowest value there. The sharp changes in 5^{o} and 15^{o} curves locate at the starting points of the tapered ends of myofibers.

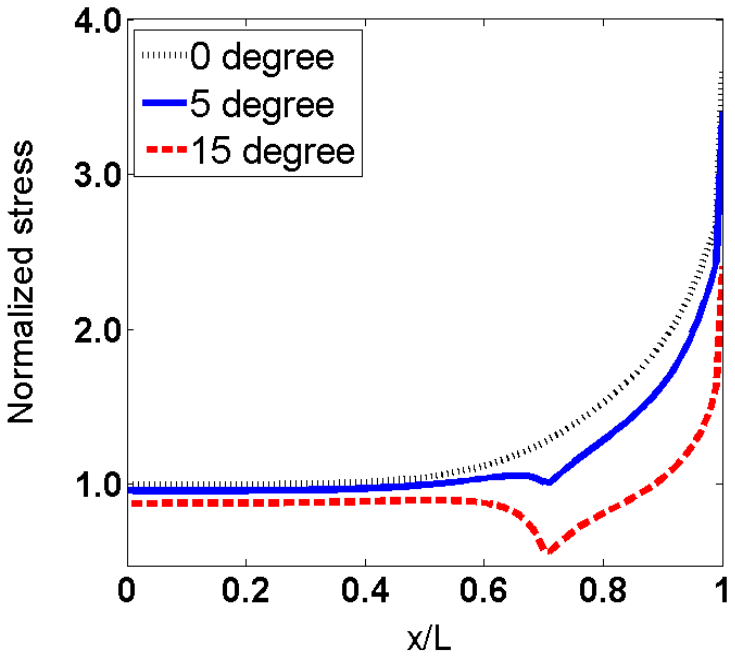

Figure 2.9 Von Mises stress distributions along myofibers with different tapered angles

2.4 Discussion

We present an FE model of a single muscle fiber that is composed of myofiber and the surrounding endomysium. The Zahalak's model was applied to simulate active force generated by the myofiber. Compared to previous studies, the present model has several advantages. First of all, unlike most previous models which considered myofibers and ECM as one material (e.g. Blemker et al., 2005; Johansson et al., 2000; Oomens et al. 2003; van der Linden et al., 1998), the present model modeled the single muscle fiber as a fiber-reinforced composite, namely, the myofiber is embedded in the endomysium, which made it possible to analyze the force transmission between myofiber and the endomysium. Secondly, although some previous FE studies also incorporated two separate materials (e.g. Yucesoy et al., 2002; Sharafi and Blemker, 2011), the myofiber and the ECM, geometrical limitations of these models restricted the force generated to be only transmitted to the ECM through shear stress, which is not consistent with experimental studies (Purslow, 2002). The geometry of our model represented a microstructure which allowed transmissions of force through both the tensile stress and the shear stress on the myofiber-endomysium interface simultaneously, by which contributions from both tensile and shear stress to the force transmission can be determined. Additionally, most of the previous FEM models (Blemker et al., 2005; Chen and Zeltzer, 1992; Chi et al., 2010; Johansson et al., 2000; Sharafi et al., 2011; van der Helm, 1994; Yucesoy et al., 2002; Zajac, 1989) used the Hill model to describe the muscle force generation, therefore, providing no information

of structural mechanism of muscle contraction. Our model combined an active force model based on biophysical and biochemical behaviors within sarcomeres during the contraction to describe the constitutive relationship of the myofiber. Injury, aging and diseases in muscle will result in changes in structures, and therefore it is desirable to develop models that include structures that affect the force generations. The active force model incorporated in the present study provided us with more control of parameters related to the fiber structure and the ECM environment, and therefore allowed us to further study the effects of injury, disease and aging on structure-function relationship of skeletal muscle in the future.

Two stress distributions along the longitudinal direction were determined in this study: tensile stress distribution in the myofiber and the interfacial shear stress between the myofiber and the endomysium. The distributions suggested that the force generated by myofiber contractions is transmitted to the endomysium by shear mainly at the end of the myofiber. Our results showed that very small interfacial shear between myofiber and the endomysium is observed in the middle of the myofibers, indicating little or no force transmission between myofiber and the endomysium happens in this region. The tensile stress dropped at the end of the myofiber and the interfacial shear stress increased simultaneously, suggesting that the tensile force is transmitted to the endomysium by shear as the endomysium is too compliant to transmit the high tensile stress. Therefore, as shown in Figure 2.5, except at the very end of the myofibers, the tensile stress is much larger than the interfacial shear stress.

Stress distributions predicted from our model are consistent with analytical analysis of 1D shear lag model (Cox, 1952), a model commonly used to analyze the stress transfer between fiber and matrix in fiber-reinforced composites. According to the shear-lag model, tensile stress is maximal in the middle of fiber and drops to zero towards the end. Shear stress in the matrix increases from zero in the middle of fibers to the maximal value at the end of fibers. In addition, our result that increasing the stiffness of the ECM would enhance the force that can be transmitted to the end of the single fiber is also consistent with the analytical analysis from the shear lag model. This result was also consistent with a recent study (Sharafi and Blemker, 2011) indicating that the total force transmitted increased with increasing ECM stiffness.

In this study, tapered ends resulted in a gradual change of geometry at myofiber ends. Smaller shear stress and von-Mises stress were observed in myofibers with larger taper angle at the end of myofiber, which suggested that the gradual changes in geometry have advantages in reducing the stress concentrations. Tidball et al. (1993) demonstrated that at the muscle level, the failure of skeletal muscles during contraction mostly took place around the myotendinous junction, and it was suggested this failure is related to the stress concentration there (Gao et al. 2008). In addition to gradual change of geometry, previous study demonstrated that there were increased folding interfaces near the tapered end of myofibers, with which the circumferential surface area of the fiber towards the end is significantly increased (Trotter, 1991; Trotter et al., 1995), which will further decrease the stress concentration at the end of myofibers.

While reducing the stress concentration at the end of the myofiber, tapered end also has an advantage in increasing the efficiency of the force transmission between the myofiber and the endomysium. In myofibers with tapered ends, the number of sarcomeres in parallel decreases along the myofiber towards the end due to reduced cross-sectional area, which in turn results in a decrease in the tensile force along the fiber (Figure 2.8). However, the loss of contractile force induced by the cross-sectional area change is compensated by transmitting the force to the endomysium laterally, and therefore, the total force transmitted to the end of the muscle fiber (the structural unit) does not decrease as much as that at the end of the myofiber. To better explain this effect, efficiency of lateral transmission of force, η_L, was introduced and defined as the ratio between the reaction force at the end and the tensile force at the end of myofiber, i.e., $\eta_L = \frac{F}{F_M|_{x=l}}$, where $F_M|_{x=l}$ is the force in myofiber at $x = l$ (Figure 2.3). Value of η_L of single muscle fiber with 0, 5 and 15 degrees of tapered angle are 1.43, 1.53and 1.87, respectively, all of which reach 90% of corresponding value of $F_M|_{x=0}$. This observation is supported by Trotter and Purslow (1995), in which the authors stated that the tapered ends in the fiber significantly increased the efficiency of force transmission through shear at the myofiber-ECM interface.

In the present study, we proved that most of the force generated is transmitted near the end of the myofiber through shear stress to the ECM, and the force transmitted to the end of the model increases with increased stiffness of the ECM. The present work

also demonstrates that the tapered angle of the myofiber end has an effect to reduce the stress concentration near the myofiber end which might lead to the injury within skeletal muscles. We showed that the mechanical mechanisms of force transmission are only affected by mechanical and geometrical properties of myofibers and the endomysium, and therefore, no differences are expected with lengthening and/or shortening contraction with no injuries occurring during the contractions. However, lengthening contractions and shortening contractions could lead to injuries in myofibers (Lieber, 2002), the endomysium and their interface, and such injuries will in turn cause changes in force transmission (Gao et al., 2008). Therefore, only the isometric contraction was studied in this present study. Effects of injury on force transmission and, in the other hand, how force transmission affects injury should both be studied in the future. To accomplish it, the myofiber-endomysium will be modeled as non-perfect bonding surface. Additionally, in the future, lateral transmission of force between muscle fibers will be studied by incorporating the present model to a three-fiber system by modifying a previously developed simplified analytical model (Gao et al., 2007). Effect of nonlinearity and time dependent properties of the ECM will then be considered in the model as it was studied previously (Gao et al., 2009).

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Chapter 3

Effects of Aging on the Lateral Transmission of Force in Rat Skeletal Muscle[2]

3.1 Introduction

By the year 2030, 20% of the population of the United States will be over 65 years old (U.S. Census Bureau, 2008). Disabilities associated with muscle weakness, known as sarcopenia, are common, and significantly influence the quality of their daily life (Morley et. al., 2001; Roubenoff, 2001; Janssen et al., 2002). The age-related reduction in muscle force has been commonly attributed to the loss of muscle mass. However, muscle force is lost to a greater extent than the loss of muscle mass, suggesting that other factors are involved (Nair, 2005).

The loss of muscle force in aged muscles is not simply caused by degeneration of myofibers. Changes in ATPase activity, metabolite levels, or myosin isoforms have been proposed to be the possible mechanisms; however, none of them can fully explain the

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loss of force in aged muscles (Philips et al., 1993**;** Lowe et al., 2001; Lowe et al., 2002). Previous studies showed that although the specific force (force per area) of the whole muscles decreases with aging (~13% - 20%), there is no significant deficit in specific force of single skinned muscle fibers between the young and old groups (Eddinger et al., 1986; Brooks and Faulkner, 1988, 1994; Philips et al., 1991). The deficit in specific force of whole muscles cannot be explained by unimpaired intrinsic force-generating capacity of cross bridges in aged muscles, suggesting that the force transmission from muscle fibers to the tendon in aged muscles could be impaired, leading to the loss of skeletal muscle strength.

Two pathways are involved in transmitting force from muscle fibers to tendon: 1) longitudinal transmission, i.e., transmission along the muscle fibers via the myotendinous junctions (MTJ) to the tendon; and 2) lateral transmission, i.e., transmission laterally across one muscle fiber to the adjacent connective tissue network, the extracellular matrix (ECM), and finally to the tendon (Huijing, 1999). The myotendinous junction has been thought to be the main site of force transmission. However, muscle fibers frequently terminate within the fascicles without reaching the MTJ for muscles across species (Gaunt and Gans, 1992; Trotter, 1993; Trotter and Purslow, 1992; Huijing, 1999). This anatomic structure suggests that the force generated in these muscle fibers has to be transmitted laterally via the ECM, and then to the tendon. Therefore, the force transmitted to the end of muscle could be significantly affected by the ECM (Zhang and Gao, 2012; Gao et al., 2009). As the stiffness and the

thickness of ECM in skeletal muscles increase with aging (Nishimura et al., 1997; Kjaer, 2004; Gao et al., 2008), we believe that lateral transmission of force could be affected due to these changes. However, previous studies by Ramaswamy et al. (2011) found that significant difference on lateral transmission only exists between the young (3 months) and very old rats (36-38 months), but not between the young and old (30-33 months). The conflicting findings motivated us to re-investigate the effects of aging on lateral transmission.

The objective of this study was to determine the effects of aging on the lateral transmission of force in skeletal muscle, and explore the potential underlying mechanisms. We hypothesized that lateral transmission pathway is impaired in aged skeletal muscle, and the impaired lateral transmission could be partly due to increased thickness of the ECM. To test our hypothesis, *in vitro* isometric contractile tests were performed on the extensor digitorum longus (EDL) muscle of young and old rats with series of tenotomy and myotomy between adjacent heads. Proportions of forces transmitted laterally and longitudinally were then calculated and compared between young and old rats.

3.2 Methods

Two groups of male Brown Norway rats were used in our experiments: young (3-4 months old, n = 6) from Harlan Laboratories (Indianapolis, IN), and old (32 months old, n = 5) from National Institutes of Aging (Baltimore, MD). All procedures used in

this study were approved by Cornell University's Institutional Animal Care and Use Committee (IACUC).

3.2.1 Experimental procedures

After anesthesia, the left extensor digitorum longus (EDL) muscle of each rat was isolated. The EDL is a multi-tendon muscle with distal insertions on digits II-V of the foot, a well-established model for characterizing the force transmission through ECM between the four muscle heads (Huijing et al., 1998; Maas et al., 2003). Silk suture was tied to the distal tendon of the muscle as proximally as possible without damaging the muscle. The suture stayed intact throughout the experiments.

After dissected free from the body, the EDL was fixed to an in vitro contractile testing system (1205A, Aurora Scientific, Toronto, ON) with the proximal end fixed by a clamp, and the suture on the distal end attached to force transducer (resolution 1.0mN). The EDL was placed in mammalian ringer's solution, and stimulated by a 100Hz, 30V stimulation signal for maximum force generation for 600ms (Brooks & Faulkner, 1988). Three minutes rest was applied between each contraction. The optimal length was then determined, and maximum isometric tetanic force generated was measured and recorded as F_0.

The distal end of the EDL was then detached from the force transducer. The tendon of head II was cut (tenotomy) without touching the suture on the tendon, and

the distal end was reattached to force transducer. The same electrical simulation was then applied to the whole muscle at the optimal length. The force measured was recorded as F_2', which is the force measured after longitudinal transmission pathway is cut off. After measuring force generated with tenotomy to head II, the distal end was detached again, and the ECM between heads II and III was cut to separate heads II and III. Force generated by the EDL with this process was measured as F_2, which is the force measured after both longitudinal and lateral transmissions were cut off for head II. We assume the difference between F_0 and F_2, i.e., $F_0 - F_2$, was the force generated by head II; the difference between F_0 and F_2', i.e., $F_0 - F_2'$ was the force generated by head II and transmitted through tendon II; and the difference between F_2' and F_2, i.e., $F_2' - F_2$, was the force generated by head II and transmitted laterally through the ECM between head II and head III. Repeated tenotomy and myotomy were then applied to heads III, IV and the interface between corresponding heads to measure F_3', F_3, F_4', and F_4, respectively.

A schematic illustration of the procedure is shown in Figure 3.1. For each force measurement, three contractile tests were taken and an average value was calculated. To reduce the geometric variances between EDLs, we normalized each measurement to the maximum force generated by the whole EDL muscle, F_0, i.e., $\alpha_i = F_i / F_0$, $\alpha_i' = F_i' / F_0$ $(i = 2, 3, \textit{and } 4)$. The force generated in each head $F_{i-1} - F_i$ $(i = 2, 3, \textit{and } 4, F_1 = F_0)$ was

divided into two parts, the force transmitted laterally ($F_i' - F_i$) and the force transmitted longitudinally ($F_{i-1} - F_i'$), and two respective ratios $\beta_i = \frac{F_i' - F_i}{F_{i-1} - F_i}$ and $\gamma_i = \frac{F_{i-1} - F_i'}{F_{i-1} - F_i}$ ($i = 2, 3, \text{and } 4, F_1 = F_0$) were calculated.

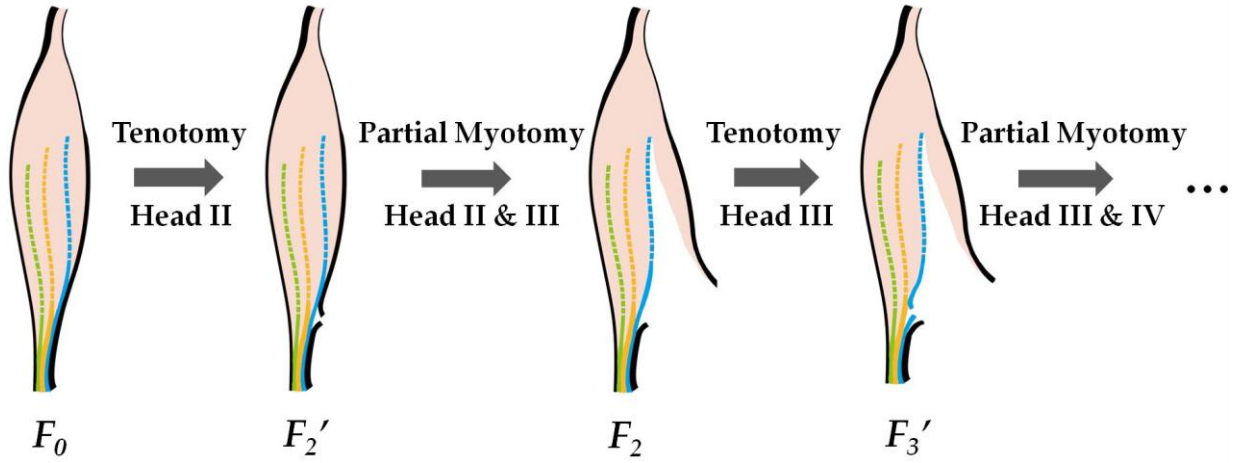

Figure 3.1 A schematic representation of contractile tests with sequential tenotomy and myotomy between different heads in EDL. First of all, maximum isometric tetanic force of the intact muscle, F_0, was measured at the optimal length. F_2' is the force measured after tenotomy of tendon of head II, and F_2 is the force measured after a partial myotomy, cutting of the ECM, between head II and head III. Same procedure was then repeated for heads III and IV.

The right EDL of each rat was dissected from rats and fixed in 10% neutral buffered formalin solution. The tissue was then fixed and cut into $3\mu m$ sections for hematoxylin and eosin (H&E) staining. ImageJ (National Institutes of Health, Bethesda, MD) was used to measure the thickness of the perimysium.

3.2.2 Statistics analysis

The variance equality and the normality of measurements were checked, and student's t-test between two groups of small samples was conducted to compare forces transmitted through the ECM between young and old groups. Differences in proportion of force transmitted laterally between young and old groups were assessed using analysis of variance (ANOVA). Difference was considered statistically significant at $p < 0.05$.

3.3 Results

The average maximum isometric force, represented by F_0, generated in young group is 2.04 ± 0.22 N, and that of the aged group is 1.60 ± 0.20 N (values are presented as mean $\pm$ s.d.). Significant difference of F_0 was found between young and old groups ($p<0.01$). Total forces transmitted to the end of the EDL in the young group during series of tenotomy and myotomy were shown in Figure 3.2a. Significant differences between α_2' and α_2, α_3' and α_3, and α_4' and α_4 were observed. There is no significant difference between α_2 and α_3', and α_3 and α_4'. Similar results were found for the aged group, which is shown in Figure 3.2b, except that α_2' and α_2 are not significantly different for EDLs in aged group.

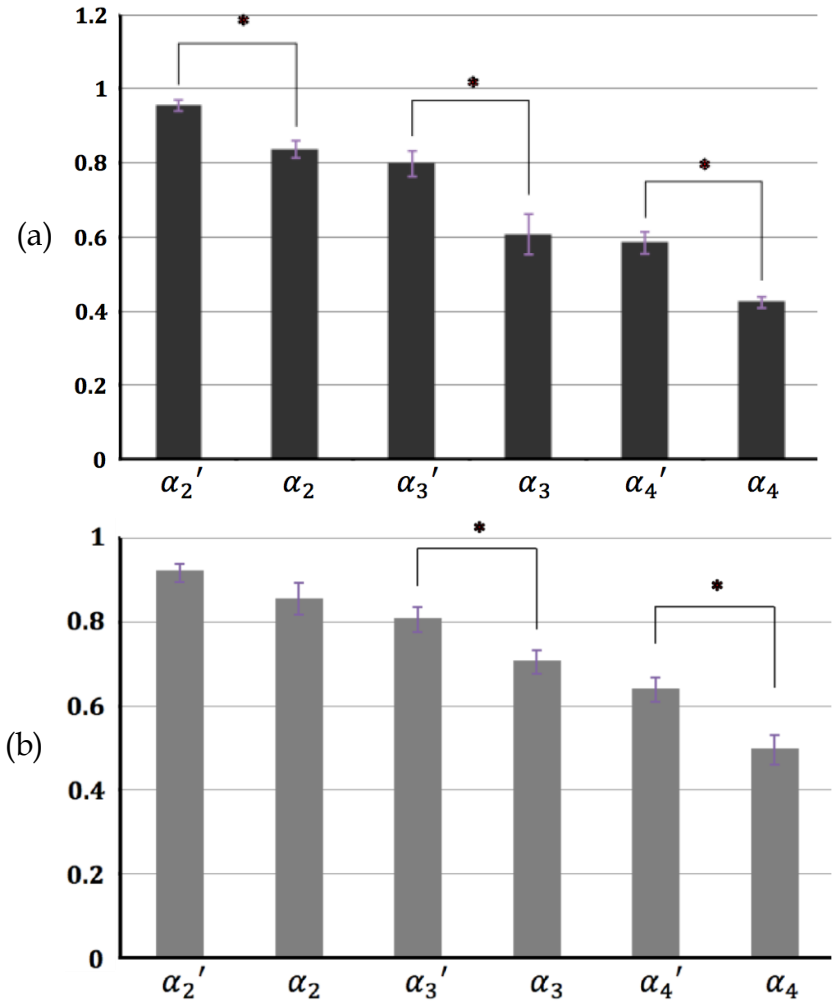

Figure 3.2 (a) Forces transmitted during series of tenotomy and partial myotomy of the young group (b) Forces transmitted during series of tenotomy and partial myotomy of the old group. ($\alpha_i = F_i / F_0$, $\alpha_i' = F_i' / F_0$ $(i = 2, 3, and\ 4)$)

As shown in Figure 3.3, there are significant differences in β_2 and β_3 between young and old groups, suggesting larger fractions of force generated in heads II and III were transmitted laterally in young muscle. This result indicates that the efficiency of lateral transmission of force in the EDL muscle is impaired in old rats. Significant

differences in γ_2 and γ_3 between young and old groups were also seen (Figure 3.4), suggesting that in heads II and III, the force that needed to be transmitted longitudinally increased in the old group. No significant difference was found in β_4 or γ_4 between two groups.

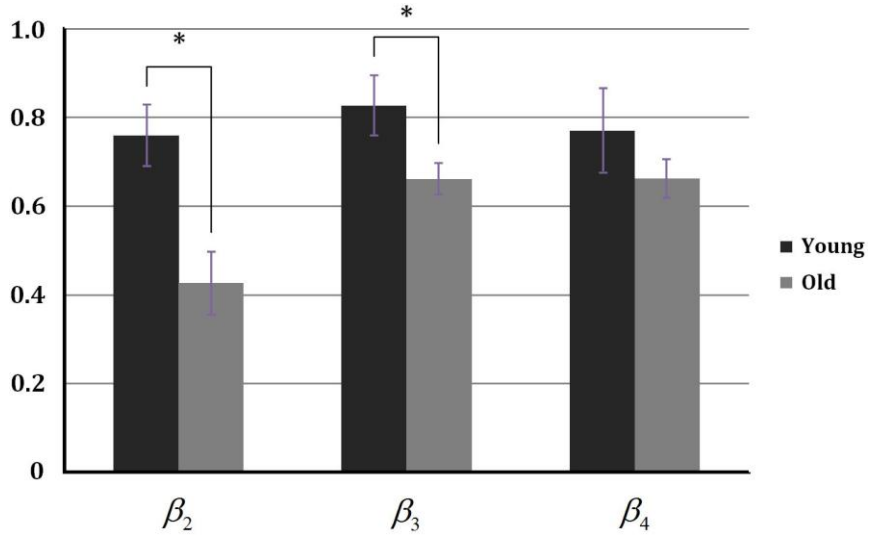

Figure 3.3 Comparison in the proportion of force transmitted laterally in each head ($\beta_i = \frac{F_i' - F_i}{F_{i-1} - F_i}$ $(i = 2, 3, and\ 4, F_1 = F_0)$) between young and old groups.

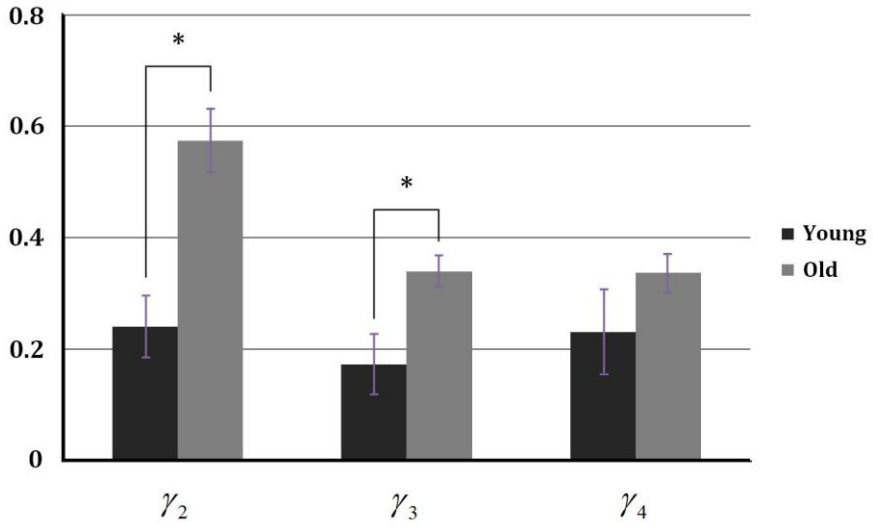

Figure 3.4 Comparison in the proportion of force transmitted longitudinally in each head ($\gamma_i = \frac{F_{i-1} - F_i'}{F_{i-1} - F_i}$ *($i = 2, 3, and\ 4, F_1 = F_0$)) between young and old groups.*

ECM thickness of the old EDL is significantly larger than that of young rats as shown in Figure 3.5. The average thickness of perimysium in the young group was $16.87 \pm 5.5 \mu m$, and that measured in the old group was $44.14 \pm 16.55 \mu m$. The average thickness of perimysium in the old group was significantly larger than that of the young group ($p < 0.0001$). In Figure 3.5, the cross section of the EDL in young rats demonstrated a more integrated structure and compact myofibers. In contrast, small segments of myofibers isolated by much thicker perimysium were seen in the EDL of aged rats.

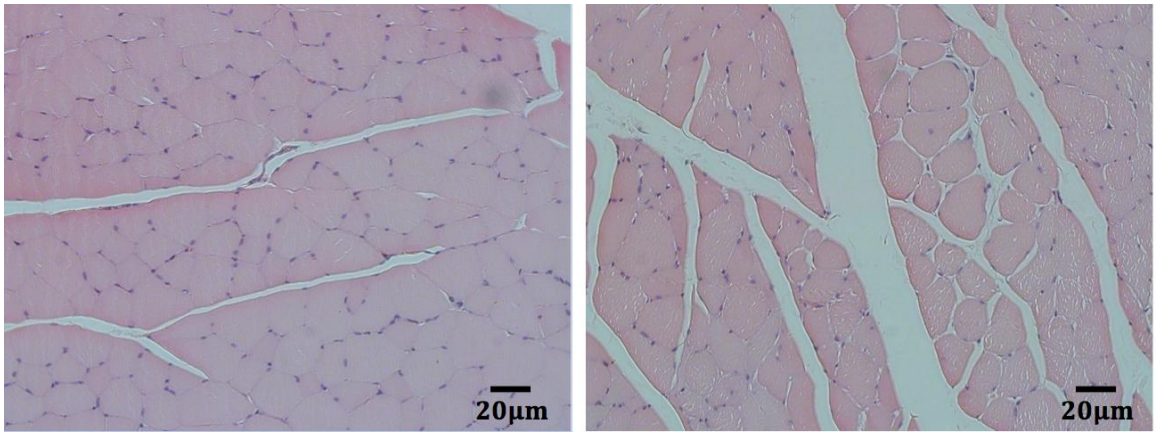

Figure 3.5 HE staining of cross-sections of right EDL of young (left) and old (right) rats.

3.4 Discussion

We demonstrated lateral transmission of force is significantly impaired in old muscle. Parametric analysis by a previous single muscle fiber model suggests that the increased thickness of the ECM induced by aging could be one of the potential compelling mechanisms that cause impaired lateral transmission in old muscles.

The role of lateral transmission through the ECM is well demonstrated in this study. This is supported by two observations in both young and old groups. First of all, no significant differences in contractile force measurement after tenotomy only (α_2 vs. α_3', and α_3 vs. α_4'). Secondly, significant reduction in force is seen after the myotomy processed at the interface, i.e., α_i' vs. α_i. This suggests that cutting the myotendinous junction only does not affect force transmission as the force can still be transmitted laterally through the ECM to the fascicles that are still connected to the

tendon. However, cutting the ECM between muscle heads after tenotomy removes the lateral pathway of force transmission, therefore, the force generated by that head of the EDL cannot be transmitted. This result is consistent with a previous study, in which lateral transmission in young rats was determined (Huijing et al., 1998). They found that cutting the longitudinal pathway for approximate 55% of the total EDL mass only caused about 15% drop on the total force transmitted to the end. However, the following myotomy at the interface between head III and head IV caused the force transmitted dropped to approximate 50% of the entire muscle.

Our experimental results suggest that the lateral transmission of force is impaired in old EDL muscle. Although cutting ECM after tenotomy caused decreased muscle force measured in both young and old groups, significant higher force reduction was observed in the young group, suggesting that more force is transferred to the tendon through lateral transmission in the young group. In addition, we measured that the total force transmitted laterally in the entire EDL decreased by 16.133% due to aging, which is consistent with previous experimental observations of about 13% - 20% deficit in aged muscles (Brooks and Faulkner, 1988; Philips et al., 1991). This finding further suggested that the impairment in lateral transmission with aging could be one of the main reasons for the decreased specific force of entire muscle.

In current study, alternately cutting the tendon and the ECM between different heads is performed. This allows us to perform parametric analysis experimentally so that we can quantitatively determine the role of each removed tendon head on

longitudinal transmission and the role of the cut ECM between each head on lateral transmission. For example, in addition to aforementioned impaired lateral transmission in old muscle, longitudinal transmission needs to play a more important role with aging, indicated by the difference on β_i and γ_i between young and old groups. For the force generated by head II and III, the proportion of force transmitted laterally (β_2 and β_3) is significantly smaller in old EDL, and as a result, the proportion of force transmitted longitudinally (γ_2 and γ_3) in each head significantly increased due to aging.

Different from the conclusion that Ramaswamy et al. (2011) made, we showed impaired lateral transmission of force in old rats of 32 months old. We believe that these two different observations are due to the following two reasons. Firstly, the yoke (Ramaswamy et al., 2011) sutured to the middle of EDL could cause damage to epimysium, which is one of the key components for lateral transmission. Secondly, in the lateral transmission measurement, the yoke instead of the distal tendon was connected to the force transducer, resulting nearly one half of the fibers, i.e., the segment between the yoke and the distal end of the muscle, tend to change from the optimal length to slack length, which would significantly affect the force measured.

Increased thickness of the ECM induced by aging could be an important reason of the impaired lateral transmission of force in old muscle. Similar to other previous studies (Alnaqeeb et al., 1984; Purslow, 2002; Fang et al., 1999), significant increase in

thickness of ECM in old rats is observed in present study. From a previously developed FE model (Zhang & Gao, 2012), we believe that increasing the thickness of ECM leads to decreased force transmitted to the end of muscle. This is consistent with a different model in which increased thickness of the ECM can result in drop of force transmitted laterally (Sharafi & Blemker, 2011). In lateral transmission, force is transferred between muscle fibers by shear stresses in the ECM. Considering skeletal muscle as a fiber-reinforced composite (Huijing, 1999; Yucesoy et al., 2002; Blemker et al., 2005; Zhang & Gao, 2012), the reduction of lateral transmission of force with thicker ECM could also be explained by shear lag model of short fiber-reinforced composite (Cox, 1952), in which states that the thicker the matrix is, the less efficient of lateral transmission is.

However, increased thickness of the ECM does not fully explain the impaired lateral transmission of force in old muscle. We only found an approximate 5% drop of force transmitted when doubling the thickness of ECM, which is much lower than 13-20% drop of force in aged muscle (Eddinger et al., 1986; Brooks & Faulkner, 1988), indicating other mechanisms are involved. The stiffness of the ECM of old muscle could increase to 1.5 – 2 times of that of the adult (Alnaqeeb et al., 1984; Gao et al., 2008), which could affect the transmission of force. In addition, according to mechanics of fiber-reinforced composite, changes in interface between the fiber and matrix could affect the force transmission between them. In muscle, dystrophin–glycoprotein complex (DGC) is one of the structures that connect the ECM and myofibers laterally. Therefore, any change in the number or type of DGC could affect the lateral

transmission. How the number and type of DGC are affected by aging is controversial and unclear. For example, Ramaswamy et al. (2011) found that number of dystrophin decreases in EDL in very old rats, and therefore, believed that aging caused significant reduction in the expression of dystrophin is correlated to the impaired lateral transmission of force. Rice et al. (2006), however, reported that the amount of both dystrophin and β-dystroglycan increased in aged EDL. They also stated that the age-related DGC change also depended on the type of skeletal muscle. Therefore, further studies on the effects of aging on changes in the molecular linkages between the ECM and the fiber, such as dystrophin, vinculin, talin, etc., are needed.

The lateral transmission of force involves very complicated mechanical interactions between muscle fiber and the ECM. Aging is associated with changes in geometrical, structural and mechanical properties of ECM, muscle fibers and the molecules in between. Experimental results can only show the overall effects of all changes in structural and mechanical properties, and the effect of each individual factor is difficult to be determined. Mathematical model has the advantage of analyzing effects of individual factor by parametric analysis. In the future, a computational model that incorporates the physiological structures and appropriate material properties will be developed. Comparing the parametric analysis of the computational model to our experimental results will allow us to identify the underlying mechanisms of impaired lateral transmission associated with aging.

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Chapter 4

The Role of Transmembrane Proteins on Force Transmission in Skeletal Muscle[3]

4.1 Introduction

The population over 65 years old in U.S. was 40.2 million in 2010, and is estimated to reach 88.5 million in 2050 (Administration on Aging, 2011). One of the major concerns to this elderly population is aging of skeletal muscle, also called sarcopenia, characterized by involuntary loss of muscle mass and strength (Morley et al., 2001). Approximately 45% of the U.S. elderly population is affected by sarcopenia (Janssen et al., 2004). The estimated annual healthcare cost directly related to aging of skeletal muscle was over $18 billion in 2004, and the number only keeps increasing with the increasing elderly population (Janssen et al., 2004).

Previous studies showed that the loss of muscle strength induced by aging is not proportional to the loss of muscle mass (Nair, 2005). Changes in ATPase activity,

3. Zhang, C. & Gao, Y., The Role of Transmembrane Proteins on Force Transmission in Skeletal Muscle (paper in preparation).

metabolite levels, or myosin isoforms have been proposed to be the possible mechanisms; however, none of them can fully explain the loss of strength in aged muscles (Philips et al., 1993**;** Lowe et al., 2001; Lowe et al., 2002). Specific force (force per unit area) of whole muscle decreases about 20% with aging; however, there is no significant difference in the specific force of single muscle fibers between young and old muscles (Eddinger et al., 1986; Brooks and Faulkner, 1988, 1994). Therefore, the overall decreased strength of skeletal muscle cannot be explained by the unaffected force generation capability of single muscle fibers, indicating that the force transmission pathway from single fibers to the end of the whole muscle is somehow impaired with aging, and the impaired force transmission leads to decreased total muscle force.

Two pathways are involved in transmitting force from muscle fiber to the tendon: the longitudinal transmission of force, in which force is transmitted through the fiber along the fiber direction, and then to the myotendinous junction between fibers and tendon and finally to the tendon; and lateral transmission of force, in which force is transmitted laterally through the membrane of the muscle cell to the extracellular matrix (ECM), and finally to the tendon. The existence of the lateral pathway has been proved by several experiments, and was considered to be able to transmit about 80% of total force transmitted (Huijing et al., 1998; Ramaswamy, 2011, Street 1983).

Although the lateral transmission is proved to be impaired with aging, the mechanisms under this phenomenon are not fully understood. One of the potential reasons could be the aging induced change in ECM, which becomes thicker and stiffer

with aging. We have shown that geometrical and mechanical properties, such as thickness and stiffness, of the ECM affect lateral transmission of force (Zhang and Gao, 2012). However, parametric analysis showed that changes on thickness and stiffness of ECM only cannot fully explain the ~20% reduction in the specific force of aged muscle (Zhang and Gao, 2012).

In addition to the ECM, the structures that are responsible for lateral transmission involve the transmembrane proteins that connect the ECM and myofiber. There are two chains of proteins that are discontinuously distributed along the interface between the myofiber and the surrounding ECM (Lieber, 2002). The first chain is called dystrophin–glycoprotein complex (DGC), in which the actin binds to dystrophin and dystroglycan in the sarcolemma, and then to the collagens in the extracellular matrix. DGC transmits forces from actin to the ECM, as well as supports muscle fiber strength and stabilize the microstructure within myofiber to prevent muscle fiber injury (Petrof et al., 1993; Kaariainen et al., 2000). In a second chain of proteins, the actin binds to the talin, which binds vinculin and then to integrin, and finally to collagen fibers in the ECM (Tidball, 1991).

Changes in number of the transmembrane proteins have been reported, although it is controversial. For example, Ramaswamy et al. (2011) found that number of dystrophin decreases in EDL in very old rats, and therefore, believed that aging induced reduction in number of dystrophin is correlated to the impaired lateral transmission of force. Rice et al. (2006) reported that the amount of both dystrophin and

β-dystroglycan increased in aged EDL, and also stated that the age-related DGC change is dependent on the type of skeletal muscle.

The objective of this study is to determine how changes in the amount (spatial density) and mechanical properties of these membrane proteins affect the lateral transmission of force in skeletal muscle. We hypothesized that increasing either the amount (spatial density) or the stiffness of the membrane proteins increase the total force transmitted in skeletal muscle. To test this hypothesis, a finite element model including the myofiber, ECM, and the transmembrane proteins is developed. Parametric analysis is performed to determine the effects of spatial density and mechanical stiffness of the transmembrane proteins on force transmission.

4.2 Methods

Skeletal muscle is functionally a fiber-reinforced composite, and the basic structural unit of muscle is a single muscle fiber (Zhang and Gao, 2012). In this current study, a finite element model of a single muscle fiber, including the myofiber, the surrounding ECM (i.e., endomysium), and the transmembrane proteins between the myofiber and the ECM was developed to determine the force transmission from myofiber to transmembrane proteins, to the ECM, and then to the end of the single muscle fiber. All the analysis is performed in ABAQUS 10.1 (Dassault Systèmes Americas Corp., Waltham, MA).

4.2.1 Model description

The model is modified from a previously developed 2D FE model, in which the single muscle fiber is modeled as one myofiber cylinder surrounded by ECM cylinder, and the interface between them was modeled as fully bonded interface (Zhang and Gao, 2012). In this study, there were discontinuously distributed transmembrane proteins between the myofiber and the ECM. Because of geometric symmetry of the structural unit, only one quarter of the single structural unit is analyzed (Figure 4.1). As shown in Figure 4.1, the myofiber ends within the model, and connects to the ECM through lateral linkages. The physiological structure of the tapered end of myofibers observed in previous studies (Barrett, 1962; Eldred et al., 1993; Gaunt and Gans, 1990; Trotter, 1990) was also incorporated into this model with a geometric simplification as a rounded corner near the end of the myofiber.

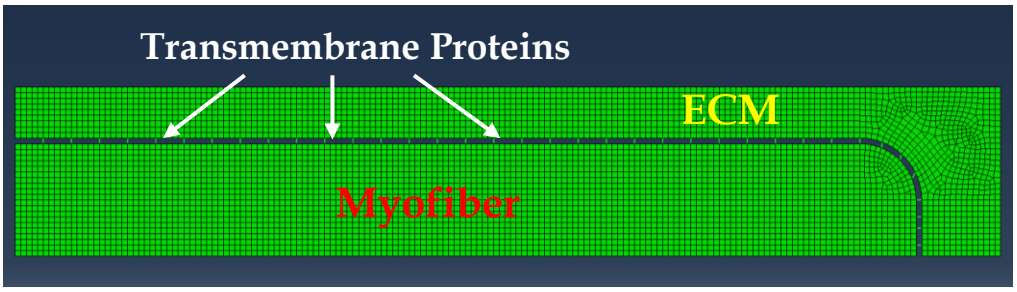

Figure 4.1 Structure of single muscle fiber model with myofiber, ECM, and transmembrane proteins (marked by arrows) along the myofiber-ECM interface.

The myofiber is modeled as nearly incompressible Mooney-Rivlin material. Active force generated in the myofiber during a contraction is calculated by

incorporating a previous developed model (Zahalak and Ma, 1990), which combines the biophysical and biochemical characteristics during the muscle contraction. The ECM is also modeled as hyperelastic material with Mooney-Rivlin properties, which is much softer than the myofiber material. Coefficients of the strain energy density function of the myofiber and ECM are determined from previous studies (Zhang & Gao, 2012; Gao et al., 2008; Sharafi & Blemker, 2011).

Previous experiments on the transmembrane proteins on force transmission pathways showed a nonlinear force-length relationship of these proteins (Bhasin et al., 2005; Garcia-Pelagrio et al., 2011). In the current study, elements representing these transmembrane proteins were modeled as nonlinear elastic components with a J-shape force-length curve modified from the previous studies (Garcia-Pelagrio et al., 2011), described as $F = 8 \cdot (\Delta L/L)^3$ (Figure 4.2).

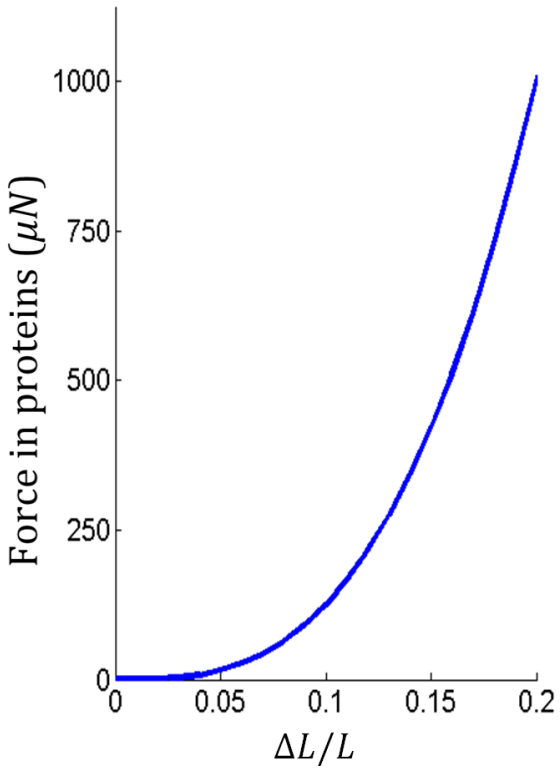

Figure 4.2 Force-length relationship of transmembrane proteins.

A 2s stimulation signal is applied to myofiber to generate an isometric contractile force. Force distribution in transmembrane proteins along the fiber direction is analyzed. Stress and strain distributions in myofiber and ECM are also determined in this model to study the effects of lateral protein change on force transmission. The efficiency of force transmission is determined in this model as the ratio between the force transmitted to the end calculated as the reaction force at the right end of muscle, and total active force generated in the myofiber.

4.2.2 Parametric analysis

Parametric analysis was conducted to determine the effects of changing in spatial density and the stiffness of the lateral linkages on the stress/strain distribution and the total force transmitted to the end. Different stiffness properties are applied to transmembrane proteins. The force-length relationship of stiff proteins is defined in Figure 4.2; the stiffness of compliant proteins is defined as 1/5 of the stiff one. A recent study found that the amount of the transmembrane proteins DGC could decrease by 60% with aging in skeletal muscle (Ramaswamy et al., 2011). To analyze the effect of spatial density of lateral linkages on force transmission, models with different linkage densities are developed as shown in Figure 4.3. The control case, in which each transmembrane protein locates at the Z-line with a sarcomere length in-between, represents the case with dense proteins. The density of proteins in the fiber with sparse proteins is 1/2 of the dense one as aging could induce more than half reduction of the transmembrane proteins (Ramaswamy et al., 2011). In addition, different levels of active force are introduced by multiplying ratios from 0 to 1 to the isometric contraction force to model cases that not all myofibers are stimulated at the same time.

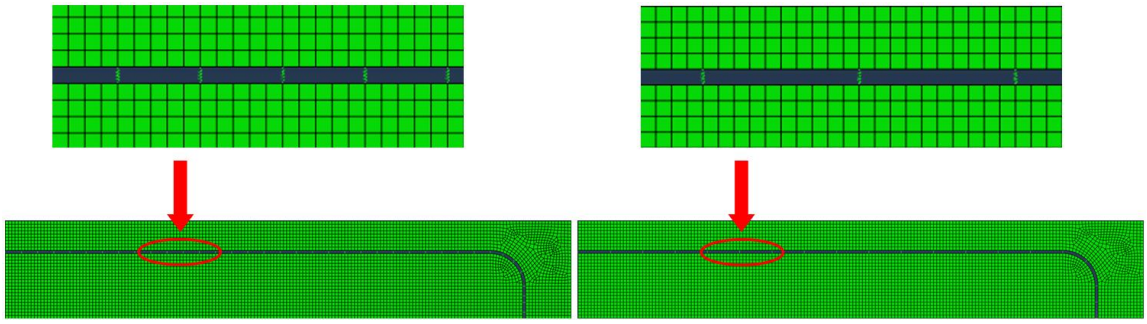

Figure 4.3 Single muscle fibers with different spatial density of transmembrane proteins. Figure on the left shows fiber with dense transmembrane proteins between myofiber and ECM; figure on the right shows sparse transmembrane proteins, which is one half of the density on the left.

4.3 Results

4.3.1 Force and shear stress distribution in transmembrane proteins

Force distribution in transmembrane proteins along the myofiber direction is presented in Figure 4.4. Forces in proteins near the center of fiber are slightly below zero, and increase gradually along the fiber direction, and get to the maximum value near the end of myofiber. The magnitude of protein force in the muscle with dense (more) proteins is lower than that of the muscle with sparse (less) proteins at corresponding locations. There is no difference in forces with stiff and compliant proteins. Shear tress distribution shows the similar trend as the force distribution in the proteins (Figure 4.5). Shear stress of myofiber at the interface in the fiber with sparse proteins has greater magnitude than that of the fiber with dense linkages, and same as the force in proteins, shear stress of myofiber at the interface are not different between

muscle fibers with stiff and compliant proteins. Similar trend of shear stress distribution is also seen in the ECM along myofiber-ECM interface.

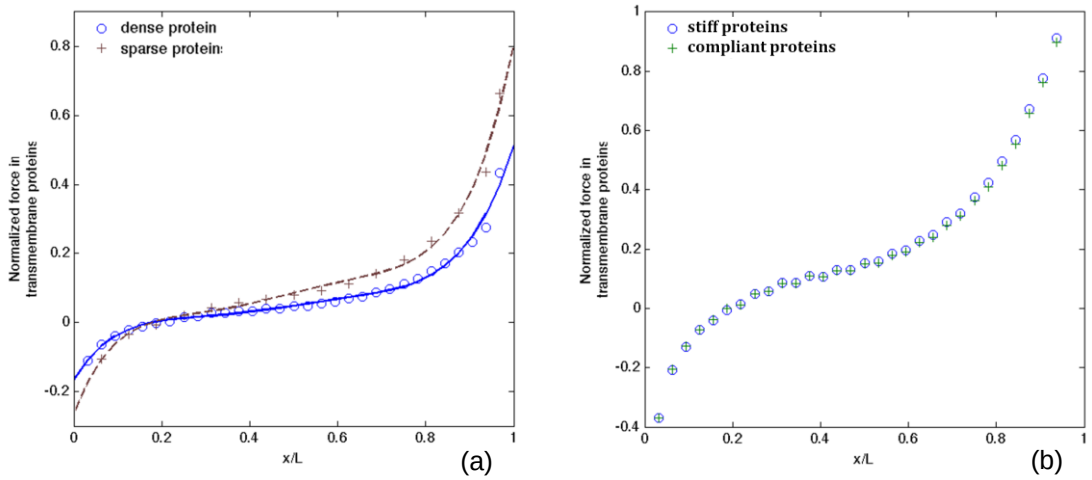

Figure 4.4 (a) Comparison of force distributions in proteins between muscle fibers with dense and sparse transmembrane proteins. (b) Comparison of force distribution in proteins between muscle fibers with stiff and compliant proteins. Y coordinates are the normalized force in the transmembrane proteins.

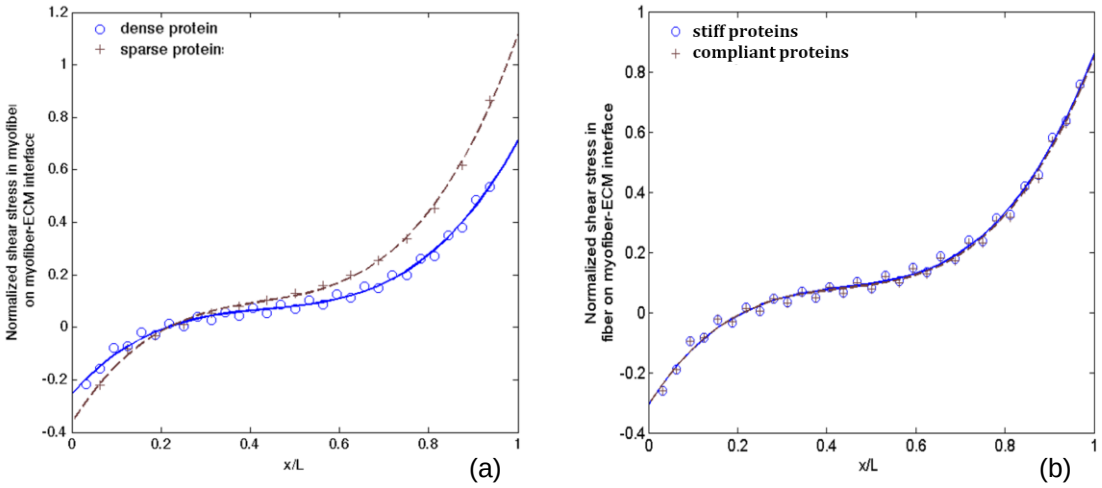

Figure 4.5 Distribution of normalized shear stress in myofiber on myofiber-ECM interface. (a) Comparison of myofiber shear stress distribution between dense and sparse proteins. (b) Comparison of myofiber shear stress distribution between stiff and sparse proteins. Each data point is calculated as an average shear stress of the two elements connecting to the transmembrane protein.

4.3.2 Force transmitted to the end of the single muscle fiber

Effect of density and mechanical properties on the efficiency of force transmission at different active force levels is shown in Figure 4.6. The force transmitted to the end decreases with larger active force in myofiber. Muscle fiber with dense proteins can transmit more force than that with sparse proteins. Surprisingly, decreasing the mechanical properties of proteins to 1/5 of the stiff ones does not affect the efficiency of force transmission (Figure 4.6b).

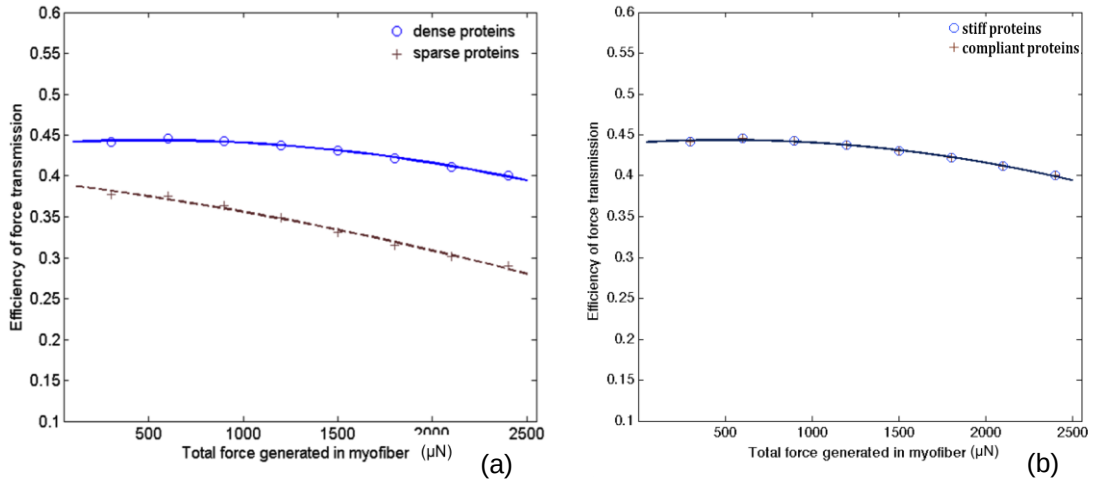

Figure 4.6 Efficiencies of force transmission of single muscle fiber at different active force generation. (a) Comparison between muscle fibers with dense and sparse proteins; (b) Comparison between muscle fibers with stiff and compliant proteins.

4.4 Discussion

The transmembrane proteins between the ECM and myofibers play an important role on lateral transmission of force in skeletal muscle. Aging, disease and injury could result in spatial density and mechanical properties changes of the membrane proteins.

However, how the transmembrane proteins transmit the contractile force to the ECM is not very well understood. In this study, a finite element model that incorporates myofiber, ECM, and the interfacial transmembrane proteins between them is developed. Parametric analysis on the spatial density and the stiffness of transmembrane proteins showed that the transmission of force is very sensitive to spatial density (also the amount) of the proteins. Changes in stiffness of the proteins do not have significant effects on the lateral transmission.

The increasing force in transmembrane proteins towards the end of myofiber suggests that the force transmission between myofiber and ECM mainly takes place near the end of myofiber. These results agree with our previous observations that the active force was found mainly transmitted near the end of myofiber through shear with a perfect bonded interface between myofiber and the ECM (Zhang & Gao, 2012). These observations are also consistent with the shear lag model theory for engineering fiber-reinforced composite materials (Cox et al., 1952).

The observation that most force generated in the myofiber is transmitted near the end of myofiber through transmembrane proteins suggests that the spatial density of proteins near the end of myofiber plays the most crucial role in force transmission. Reduction in the amount of proteins near the middle of myofiber could not affect the force transmission as much as a change of density near the end of myofiber. This observation suggests that it is important to identify the location of the transmembrane proteins. Characterizing the transmembrane at different location could be the reason for

inconsistent findings on the effect of aging on the amount of transmembrane proteins in skeletal muscle.

The spatial density change in the transmembrane proteins has significant effects on the force transmitted to the end and the force in the proteins. Insufficient amount of transmembrane proteins induces a decreased force transmitted to the end as shown in Figure 4.6. In addition to transmit more force, lower force in the transmembrane is also observed in the muscle fiber with dense (more) transmembrane proteins. With less transmembrane proteins, higher forces are also observed in the ECM and the myofibers (Figure 4.5), suggesting possible stress concentrations on the myofiber, the ECM and the interfacial transmembrane proteins in between near the end of myofiber. The stress concentrations may therefore induce injury to the end of the muscle fiber. This agrees with experimental observations that lengthening contraction-induced injury often occurs at the end of fiber (Tidball et al., 1993).

Effects of changing in spatial density of proteins on force transmission also suggest that dystrophin and dystrophin-associated proteoglycans have the function to stabilize the microstructure within myofiber to prevent muscle fiber injury (Macpherson et al. 1997; Panchangam et al. 2008). With sufficient density of the transmembrane proteins, force generated in each small section along the myofiber can be transmitted to the nearest lateral protein without any accumulated effect, resulting in a relatively lower distributed force and but more force transmitted to the end.

The result that change in the amount (density) of transmembrane proteins has

significant effects on the force transmitted to the end explains the loss of force in muscle dystrophy. In muscle dystrophy, the lost of dystrophin and dystrophin-associated proteoglycans results in much less lateral transmission of force from myofiber to the ECM, and therefore, the force transmitted to the end. Aging induced changes on the amount of membrane proteins could also affect lateral transmission. However, how the number and type of DGC are affected by aging is controversial and unclear. Ramaswamy et al. (2011) found decreased number of dystrophin in EDL in very old rats; Rice et al. (2006), however, reported that the amount of both dystrophin and β-dystroglycan increased in aged EDL, and also stated that the age-related DGC change also depended on the type of skeletal muscle. To determine the mechanism of the age related change, future work of accurate measurement of related proteins during aging is needed.

We found that the efficiency of force transmission decreases with increased active force generation. This is because of the nonlinear stress-strain relationship of the transmembrane protein. As shown in Figure 4.2, the stiffness of the proteins increases as the strain increases. As the active force increases, the stiffness of proteins increases and become much larger than that of ECM in the end. Therefore, the ECM is too compliant to transmit the large active force generated in the myofiber proportionally.

The nonlinear stress-strain relationship also explains why the efficiency of force transmission is not sensitive to the stiffness of transmembrane proteins. For example, if the stiffness of the proteins is 20% of the stiffer ones, with linear stress strain

relationship, in order to transmit the same amount of force, five times of deformations in the compliant proteins are expected compared to the stiffer ones. However, as the stiffness of the proteins increases with increased strain (Figure 4.2), to transmit the same amount of force requires much less deformations, for example, less than twice the strain of the stiffer ones. In addition, as the myofiber is much longer than the transmembrane proteins (about 200 times of the proteins), even very large changes on the strains of proteins (Figure 4.7a) will not introduce significant changes in the strains of myofiber (Figure 4.7b), and therefore, does not change the efficiency of force transmission in muscles with compliant proteins. The observation that strains in compliant proteins are significantly larger than strains in stiff proteins (Figure 4.7a) also explains why muscle fiber with compliant proteins can generate the same force as the muscle fiber with stiffer proteins (Figure 4.4b).

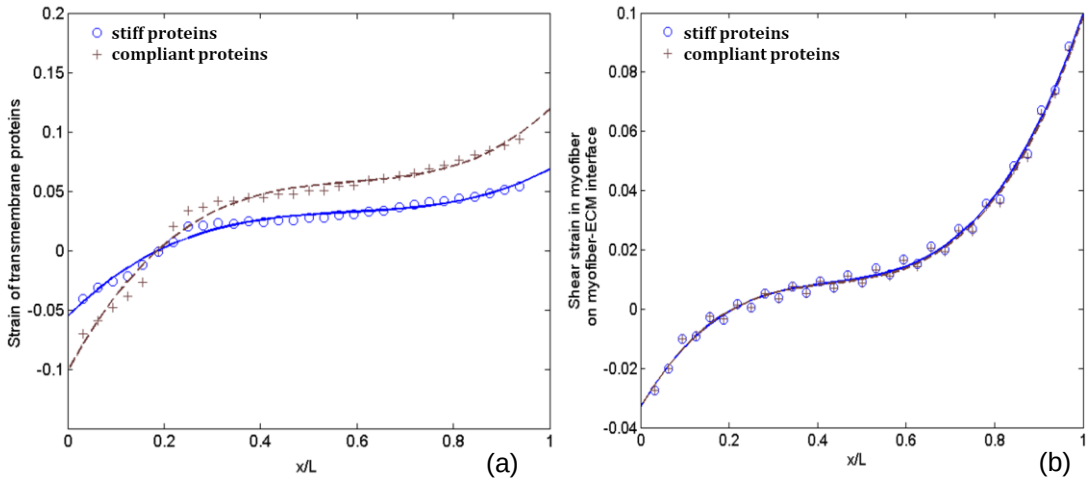

Figure 4.7 (a) Comparison of strains in proteins between muscle fibers with stiff and compliant proteins. (b) Comparison of strains in myofibers between muscle fibers with stiff and compliant proteins.

The study model the single muscle fiber using simplified geometry and mechanical properties of the ECM and transmembrane protein due to lack of appropriate experimental results. Therefore, this is a qualitative analysis rather than a quantitative one. Despite this, the study still allows us to understand the effects of changes on amount and mechanical properties of transmembrane proteins on force transmission. In the future, model with detailed structure of skeletal muscle need to be determined through experiments and image analysis. Comprehensive properties at different level of ECM also need to be measured to construct a more realistic model.

4.5 Conclusion

In conclusion, we demonstrated that the muscle with insufficient transmembrane proteins transmitted less force than muscle with more proteins. The high stress concentration at the end of myofiber with fewer proteins could cause injury of myofiber or separation between ECM and myofiber. Stiffness change of transmembrane proteins does not affect the force transmission significantly due to the nonlinear force-length relationship of these proteins, and the much smaller length of the transmembrane protein comparing to the length of the myofiber.

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Chapter 5

Conclusion and Future Work

5.1. Conclusion

Over the past decades, the elderly population that is over 65 years old has increased significantly. That population in United States was 40 million in the year 2010, and is estimated to reach 88.5 million in 2050. Diseases and disabilities related to the aging of skeletal muscle, which includes involuntary loss of muscle mass and strength, problems with mobility, falls and fractures, and even diabetes, are of excessively important concerns. Retaining the independence and physical function not only can secure the quality of life of the elderly individuals but also will save great amount of health care cost. A 10% reduction in sarcopenia would save $1.1 billion annual health cost of the states (Janssen et al., 2004). Although the muscle change due to aging is inevitable, therapies and exercise that can delay or even reverse these effects are promising (Lynch, 2011). Studies on aging of skeletal muscles is of great importance to understand the mechanisms of this age related change, and provide promising suggestions on clinical therapies, exercising and nutrition treatment. The aim of this study is to determine the mechanisms of aging induced reduction in the strength of skeletal muscle.

The results of this study indicate impaired lateral transmission contributes to aging induced muscle weakness. Lateral transmission of force in myofiber to the ECN often occurs at the end of myofiber. The force transmitted to the end of the fiber is affected by mechanical and geometrical properties of myofibers, ECM, and the transmembrane proteins at the myofibers-endomysium interface.

Chapter 2 is aimed to determine mechanical mechanism of lateral transmission of force in skeletal muscle. In this chapter, the skeletal muscle was modeled as a fiber-reinforced material with relative strong muscle fibers embedded in relative soft extracellular matrix (Huijing, 1999). A model of the basic unit of this structure, which includes one myofiber and the endomysium surrounding it, is constructed. Force transmission between the myofiber and surrounding endomysium mainly happens near the end of myofiber through shear. Increasing the stiffness of ECM alone could increase the total force transmit to the end of the single muscle fiber. Results of Chapter 2 also demonstrate that the tapered angle of the myofiber end has an effect to reduce the stress concentration near the myofiber end which might lead to the injury within skeletal muscles.

Chapter 3 is focused on the effect of aging on the force transmission pathway. We hypothesized that the lateral transmission of force is impaired during the process of aging. Experiments that measure the force transmitted longitudinally and laterally in the EDL muscle were conducted between young and old rats. The first thing we checked is the total force transmitted to the end of muscle. Significant decrease in total

force transmitted was found in the old muscle. Secondly, through series cutting of tendon connected to muscle head and ECM between muscle heads, we measured the force transmitted longitudinally and laterally respectively within each head of EDL muscle. Results showed a large proportion of force generation is transmitted laterally in muscles of young rat, however, that force decreased significantly in muscles of old rats. The force transmitted laterally decreases by ~16%, which is consistent previous literatures which found a 13-20% decrement of specific force in aged muscle. In addition, the percentage of force transmitted laterally to the total force transmitted decreased in the old muscle, proved our hypothesis that the lateral transmission of force in skeletal muscle is impaired with aging, and could be the direct reason of the loss of muscle strength. Significant increase in the thickness of ECM was observed in the aged muscle, however, parametric analysis on the thickness of ECM using the model developed in Chapter 2 showed that thickness change of ECM could not fully explain force transmission change with aging, suggesting other changes in muscle with aging is also involved.

Based on the findings in Chapter 3, Chapter 4 is focused on the effects of transmembrane proteins on force transmission in skeletal muscle. A model of single muscle fiber including the transmembrane proteins between myofiber and ECM was developed based on the previous fully bonded model. Although the age-induced change in transmembrane proteins is still unclear, some literature suggested that one kind of transmembrane proteins, the DGC, decreased with aging (Ramaswamy et al.,

2011). We hypothesized that decreases in the amount (spatial density) and stiffness of transmembrane proteins result less force transmitted to the end. The nonlinear force-length relationship of transmembrane proteins is determined from previous literatures (Bhasin et al., 2005; Garcia-Pelagrio et al., 2011). Force transmitted and forces in proteins are compared between muscle with dense and sparse density of proteins, also between stiff/weak proteins. Results showed that the muscle fiber with sparsely distributed proteins transmit lower force to the end of muscle fiber than that with the densely distributed proteins. In addition, larger force in the proteins that are sparsely distributed than those more densely distributed is observed, which is caused by the insufficiency of proteins to transmit the force generated. The high force could introduce stress concentration at the end of myofiber, causing injury to both myofiber and ECM. Surprising, muscle fiber with weak proteins transmitted the same amount of force with the stiff ones. This observation can be explained by the nonlinearity of the force-length relationship of transmembrane proteins as discussed in Chapter 4.

5.2. Future Work

This thesis raises many more questions than it answers. To further understand the mechanisms of force transmission change with aging, several important and interesting topics need to be pursued in the future: 1) an understanding of effects of aging on the material properties of endomysium and perimysium; 2) the change in the amount of transmembrane proteins with aging; and 3) techniques to build a

mathematical model of skeletal muscle, in which not only a more realistic geometry is incorporates, but also the interactions between myofiber and the ECM are considered.

First, force generated in myofiber need to be transmitted through ECM and finally to the end of muscle. Thus, the material properties of different levels of ECM structures play an important role in the force transmission. Determine the mechanical property change of ECM during aging is of great importance to understanding force transmission change during aging. Previous study on the epimysium showed that epimysium changed stiffer with aging (Gao et al., 2008), however, no material properties, especially the shear properties, have been measured directly for endomysium, which is the finest layer of ECM surrounding the myofiber. Typical testing methods are hard to separate the endomysium and test it without damage due to its tiny scale. In the future, techniques of separating single muscle fibers should be developed to measure the properties of endomysium through stretching two fibers that are connected through endomysium.

Secondly, although the results in Chapter 4 show that the force transmitted to the end of muscle is sensitive to the change of the amount of transmembrane proteins; however, how this amount is affected by aging is still controversial and unclear. Ramaswamy et al. (2011) found that number of dystrophin decreases in EDL in very old rats, Rice et al. (2006), however, reported that the amount of both dystrophin and β-dystroglycan increased in aged EDL, the same muscle. To determine whether or not the decreased force is caused by the change of transmembrane proteins, further studies on

the effects of aging on the molecular linkages between the ECM and myofibers are needed.

Additionally, to move towards determining the mechanisms of aging induced changes in force transmission, a more detailed physiologically realistic structure of skeletal muscle is needed. Most previously developed muscle models that have a relative accurate overall geometry only considered the muscle in a lump as one material, in which no transmission of force could be studied. In the future, a mathematical model of muscle with fine structures of myofiber and ECM in it should be developed. The model or the structure could be determined through cross section images of skeletal muscle and the statistical distribution of fiber length in skeletal muscle in the future.

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APPENDIX

Active stress component

The active force generated during muscle fiber contraction is calculated by using Zahalak's constitutive relations of skeletal muscle (Zahalak, 1981; Zahalak et al., 1990) which was developed from the Huxley's two-state cross-bridge theory. This model incorporated both biophysical and biomechanical processes during muscle fiber contractions.

The two-state cross-bridge theory was proposed by A.F. Huxley (Huxley, 1957). Huxley's model is based on the assumption that each cross-bridge is able to attach elastically to the actin filament close to it. Force generated by attached cross-bridge is proportional to the distance x which refers to the distance from an unstrained equilibrium position to the attached position. Define $n(x,t)$ as the fraction of bonded cross-bridge density with distance x, and taking material derivative to $n(x,t)$ gives:

$$\frac{Dn}{Dt} = (\frac{\partial n}{\partial t})_x - v(t)(\frac{\partial n}{\partial x})_t \tag{A.1}$$

where $v(t)$ is the relative shortening velocity between actin and myosin. The two-state cross-bridge theory is then described as:

$$\frac{\partial n}{\partial t} - v(t)\frac{\partial n}{\partial x} = f(x)(1-n) - g(x)n \tag{A.2}$$

in which $f(x)$ and $g(x)$ are the net attachment and detachment rates of bonded cross-bridge, respectively. In Eq. (A.2), during muscle contraction, the change of

bonded cross-bridge density with respect to time, which is shown on the left side, equals to the net attached and detached cross-bridge during this process. Therefore, Eq. (A.2) represents the dynamics equation of bonded cross-bridge density distribution during muscle contraction.

Zahalak has modified the basic Huxley model by incorporating the effect of calcium concentration on the attachment process of cross-bridges. The Ca^{2+} ions play an important role in the formation of bonded crossbridge. By considering the effect of Ca^{2+} ions, the two-state cross-bridge theory is modified as:

$$\frac{\partial n}{\partial t} - v(t)\frac{\partial n}{\partial x} = r([Ca])f(x)(1-n) - g(x)n \tag{A.3}$$

where $r([Ca])$ is a function of sarcoplasmic free calcium concentration. The free calcium concentration $r([Ca])$, is determined by the kinetic equilibrium of the chemical changing of Ca^{2+} during contraction, which is given by:

$$r([Ca]) = \frac{k_1^2[Ca]^2}{k_1^2[Ca]^2 + k_1 k_{-1}[Ca] + k_{-1}^2} \tag{A.4}$$

in which $[Ca]$ is the concentration of free Ca^{2+} ions in the sarcoplasm outside the sarcoplasmic reticulum. k_1 and k_{-1} are the calcium binding and release rates, respectively.

Therefore, force generated by cross-bridge with equilibrium distance *x* equals to:

$$k \times x \times n(x,t)$$

where k represents an elasticity constant, and total force generated within this

sarcomere is:

$$\sigma(t) = \frac{msk}{2l} \int_{-\infty}^{\infty} x \cdot n(x,t) dx \qquad \text{(A.5)}$$

where m represents the number of crossbridges per unit volume, s is the current sarcomere length, and l represents the distance between successive actin binding sites. A distributed-moment method is proposed by Zahalak et al. (1981, 1990) to solve this active stress during contraction.

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