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The Role of Transmembrane Proteins on Force Transmission in Skeletal Muscle

Chi Zhang^a and Yingxin Gao^{a,*}

^aSibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca, NY, USA

Abstract

Lateral transmission of force from myofibers laterally to the surrounding extracellular matrix (ECM) via the transmembrane proteins between them is impaired in old muscles. Changes in geometrical and mechanical properties of ECM of skeletal muscle do not fully explain the impaired lateral transmission with aging. The objective of this study was to determine the role of transmembrane proteins on force transmission in skeletal muscle. In this study, a 2D finite element model of single muscle fiber composed of myofiber, ECM, and the transmembrane proteins between them was developed to determine how changes in spatial density and mechanical properties of transmembrane proteins affect the force transmission in skeletal muscle. We found that force transmission and stress distribution are not affected by mechanical stiffness of the transmembrane proteins due to its non-linear stress-strain relationship. Results also showed that the muscle fiber with insufficient transmembrane proteins near the end of muscle fiber transmitted less force than that with more proteins does. Higher stress was observed in myofiber, ECM, and proteins in the muscle fiber with fewer proteins.

Keywords

Lateral Transmission; Myofiber; Extracellular Matrix of Skeletal Muscle; DGC

1. Introduction

Two pathways are involved in transmitting force from muscle fibers to the tendon: the longitudinal transmission, in which force is transmitted through the myotendinous junctions; and lateral transmission, in which force is transmitted laterally from myofibers to the extracellular matrix (ECM), and then to the tendon (Street 1983; Huijing et al., 1998; Gao et al., 2008b; Zhang and Gao 2012). Lateral transmission is impaired with aging, leading to decreased specific force (force/area) of whole muscle (Zhang and Gao, 2012; Zhang and Gao, 2014). The mechanisms causing the impairment are not fully understood (Zhang and

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*Corresponding author at: Sibley School of Mechanical and Aerospace Engineering, 220 Upson Hall, Cornell University, Ithaca, NY 14853. Phone: (607)255-1783, yg75@cornell.edu.

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Gao, 2014). Although aging induced changes in ECM contribute to impaired lateral transmission, they do not fully explain the ~20% reduction in specific force of aged muscle (Zhang and Gao, 2012).

The other structure that is responsible for lateral transmission is transmembrane proteins that connect the ECM and myofiber. Two chains of proteins are discontinuously distributed along the interface between the myofiber and the surrounding ECM (Lieber, 2002). In the first chain of dystrophin–glycoprotein complex (DGC), the actin binds to dystrophin and dystroglycan in the sarcolemma, and then to the collagens in the ECM. In the second chain of proteins, the actin binds to the talin, which binds to vinculin and then to integrin, and finally to collagen fibers in the ECM (Tidball, 1991).

The objective of this study is to determine how changes in spatial density and mechanical properties of the transmembrane proteins affect the lateral transmission of force in skeletal muscle. We hypothesized that increasing either the spatial density or the stiffness of the transmembrane proteins increases the force transmission. To test this hypothesis, a finite element model including the myofiber, ECM, and the transmembrane proteins is developed for parametric analysis of spatial density and mechanical stiffness of the transmembrane proteins.

2. Methods

2.1 Model description

The model is modified from a previously developed 2D FE model of a single muscle fiber (Zhang and Gao, 2012). Skeletal muscle has been viewed as a fiber-reinforced composite (Huijing 1999), in which the mechanism of lateral transmission is usually analyzed in one structural unit (Cox et al., 1952). The basic structural unit of muscle is a single muscle fiber (Gao et al., 2007; Gao et al., 2008b; Gao et al. 2009; Zhang and Gao, 2012). In this study, the single muscle fiber is modeled as a myofiber cylinder surrounded by an ECM cylinder. The transmembrane proteins were uniformly distributed along the myofiber-ECM interface non-continuously. The axisymmetric approach is used to simplify the structure of the single muscle fiber to a 2D geometry (Fig. 1a). To save the computational time, only the upper right quarter of the model was analyzed with the geometric symmetry of this model (Fig. 1b) (Zhang and Gao, 2012). 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.

A 600ms electrical stimulation signal is applied to the single muscle fiber to induce isometric contraction (Fig. 2a). The stress tensor in the myofibers (S_{ij}) is calculated as the summation of active stress due to contraction (S_{ij}^{act}) and the passive stress due to mechanical deformation (S_{ij}^{pas}), i.e. $S_{ij} = S_{ij}^{act} + S_{ij}^{pas}$. The S_{ij}^{act} is calculated as a function of cross-bridge attachment rate, calcium concentration, and contractile velocity (Zahalak and Ma, 1990). The active force generation is then incorporated in the model by applying the active force generated in each sarcomere to the Z-line along the myofiber. Both the passive

part of the myofiber and the ECM are modeled as Mooney-Rivlin 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).

Transmembrane proteins were modeled as nonlinear elastic components with a J-shape force-length curve modified from the previous studies (Bhasin et al., 2005; Garcia-Pelagrio et al., 2011), described as $F=8(\Delta L/L)^3$. Transmembrane proteins were model as geometrically discrete SPRINGA elements between the myofiber and the ECM as the dystrophins are non-continuously located at the Z-lines at the myofiber-ECM interface (Peri et al., 1994). As isometric contractions of muscle fiber usually do not induce injury (Järvinen et al., 2005), no de-bonding between the transmembrane proteins and the ECM or myofibers was considered.

Force distribution in transmembrane proteins and stress distributions in myofiber and ECM are determined. The force transmitted to the end of the fiber was calculated as the reaction force at the right end of muscle, i.e., F (Fig.2b). The efficiency of force transmission is calculated as the ratio $F/F_a|_{x=0}$ (Fig. 2b) (Zhang and Gao, 2012).

2.2 Parametric analysis

To analyze the effect of spatial density of the transmembrane proteins on force transmission, single muscle fiber with different densities and stiffness are analyzed and compared:

- Control density: the transmembrane proteins are located at the Z-lines, with a sarcomere length in between (Peri et al., 1994).
- Sparse: The density of proteins in the fiber with sparse proteins is 1/2 of the control, i.e., locates at the Z-line with two sarcomeres in between, as aging could induce more than half reduction of the transmembrane proteins (Ramaswamy et al., 2011).
- Control stiffness: the force-length relationship is described by $F=8(\Delta L/L)^3$
- Compliant: The force-length relationship of the compliant proteins was set to be

$$1/5 \text{ of the control and defined as } F=\frac{8}{5}(\Delta L/L)^3.$$

The density and stiffness of the proteins are first changed uniformly along the myofiber (Fig. 3). In addition, our previous studies suggested that force transmission between myofibers and the ECM mainly occurs near the end of the myofiber (Gao et al., 2007; Gao et al., 2008b; Zhang and Gao, 2012). To determine the effects of locations of proteins on force transmission, we also changed density or stiffness of the proteins in the middle only or near the end only. The middle 70% of the myofiber length were considered to be the middle portion and the rest of the 30% to be the region at the end. Such division is based on our previous observation that force transmission occurs at around 70% of the myofiber length (Zhang and Gao, 2012).

In addition to the density and stiffness of the transmembrane proteins, different levels of active force are introduced by multiplying ratios from 0 to 1 to the maximum isometric contraction force to model the cases when not all myofibers are activated.

3. Results

3.1 Stress distributions and force transmission with uniformly distributed transmembrane proteins

The force transmitted to the end decreases with increased active force in myofiber. Muscle fiber with control proteins transmits more force than that with sparse proteins does (Fig. 4a). The magnitude of the force in the transmembrane proteins in the single muscle fiber with more proteins (Control density) is lower than that of the muscle with fewer (Sparse) proteins at corresponding locations (Fig. 5). The myofiber-ECM interfacial shear stress at the myofiber side shows a similar trend as the distribution of the force in transmembrane proteins (Fig. 6).

However, decreasing the mechanical properties of proteins to 1/5 of the control stiffness uniformly does not affect the efficiency of force transmission (Fig. 4b), and forces and stresses in myofiber, the ECM and the interfacial proteins.

3.2 Stress distribution and force transmission with non-uniformly distributed transmembrane proteins

As shown in Fig. 7, decreasing the density of the proteins to half of the control at the end of the myofiber results much higher force in the proteins at the end of the myofibers, but no changes in the proteins in the middle. Same trend is observed for the myofiber-ECM interfacial shear stress. Decreasing the density in the middle does not affect the force transmitted to the end; however, decreasing the proteins density at the end to the half results in significant drop in force transmission (Fig. 8). Changing the stiffness of the proteins in the middle or the end does not affect the force transmission or stress distribution.

4. Discussion

The objective of this study is to determine how changes in spatial density and mechanical properties of the transmembrane proteins affect the lateral transmission of force in skeletal muscle. We found that force transmission is not affected by mechanical stiffness of the transmembrane protein due to its non-linear stress-strain relationship. The spatial density of proteins near the end of myofiber plays the most crucial role in force transmission as the contractile myofiber force is mainly transmitted to the ECM at the end. This is confirmed by the observation that reduction in the amount of proteins near the middle of myofiber does not affect the force transmission as much as decreasing density near the end of myofiber does (Fig. 8). This observation suggests that it is important to identify the location while determining the density of the transmembrane proteins. Characterizing the transmembrane at different location could be the reason for inconsistent findings on the effects of aging on the amount of transmembrane proteins in skeletal muscle in literature.

The observation that insufficient amount of transmembrane proteins at the end of the myofiber induces a decreased force transmission (Fig. 4 and Fig. 8) also partially explains less force generation in muscle dystrophy. Aging induced changes on the amount of transmembrane proteins could also affect lateral transmission. Although it is controversial on how the number and type of DGC are affected by aging, it is generally agreed that aging

could result in changes in amount and type of dystrophins (Ramaswamy et al. 2011; Rice et al., 2006). Increased interfacial shear stress and higher force in the proteins with insufficient proteins (Fig. 7) also suggest possible stress concentrations on the myofiber, the ECM and the interfacial transmembrane proteins near the end of myofiber, which may in turn cause injury. This agrees with experimental observations that lengthening contraction-induced injury often occurs at the end of fiber (Tidball et al., 1993). This result also suggests 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).

Several assumptions were made in this study. First of all, no de-bonding between the transmembrane protein and the ECM or the myofiber was considered in the model. As isometric contractions usually do not induce injury, it is reasonable to assume that no tearing (i.e., de-bonding) at the interface happens. Although de-bonding could happen with decreased amount and stiffness of the proteins, to be consistent with the control case, we assumed no de-bonding happens so that it is easier to make comparison. Secondly, continuous approach is used to model the stress-strain relationship of the proteins, which is often physiologically characterized by piecewise functions due to folding and unfolding of the protein with external loading. However, within a small range of the strain, corresponding to the first cycle of unfolding, the stress strain relationship is continuous (Gosline et al., 2002, Garcia-Pelagrio et al., 2011).

The study models the single muscle fiber using simplified geometry and mechanical properties of the ECM and transmembrane protein due to lack of appropriate experimental results. To fully capture all the morphological and mechanical characteristics of each component in single muscle fiber requires significant amount of computational time and experimental results, many of which are not available now. The simplification of the model allows us to identify the critical factors that affect the force transmission, which can be used to optimize the experimental design. We also recognized that selection of mechanical and geometrical parameters used in the model affects the stress distribution and force transmission (Gao et al., 2009; Sharafi and Blemker, 2011; Zhang and Gao, 2012; Zhang and Gao, 2014). However, the aim of the study is not to predict the exact value of the force that can be transmitted to the end; instead, it is to understand the change of trend in force transmission with decreased density or stiffness of proteins.

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Fig. 1.

(a) Schematic diagram of 2D model of the single muscle fiber. The single muscle fiber is modeled as the myofibers cylinder surrounded by the endomysium cylinder. The transmembrane proteins are uniformly distributed along the myofiber-ECM interface connecting the myofibers and the ECM laterally. Axisymmetric condition is considered, and therefore, the model is simplified to a 2D model, (b) Because of the symmetry, only the upper right quarter of the single muscle fiber is modeled. The length of myofibers is $2L$. Modified from Zhang and Gao (2012).

Fig. 2.

(a) Boundary conditions of the model. The x and r axes represent the axial and radial directions of the muscle fiber, respectively. The right end of the muscle fiber has boundary condition of $u_x = 0$ because of isometric contraction. With the symmetry, at $r = 0$, $u_r = 0$, and at $x = 0$, $u_x = 0$. The outer surface of the single muscle fiber has boundary condition of $u_r = 0$ assuming that the adjacent fibers prevent the movement along the **radial direction**, (b) The free body diagram of the model during the isometric contraction. F is the total force transmitted to the end of the single muscle fiber, and is calculated as reaction force at the right end. F_M and F_E are the forces in myofibers and the ECM, respectively. Equilibrium in force requires that $F_M + F_E = F$ at any cross-sections along the fiber direction, F_M is the summation of the active (F_a) and passive (F_p) forces in myofibers, i.e., $F_M = F_a + F_p$. The efficiency of lateral transmission of force is defined as $F/F_a|_{x=0}$. Modified from Zhang and Gao (2012).

Fig. 3. Single muscle fiber with different spatial densities of transmembrane proteins with (a) control density and (b) sparse density, defined as one half of the control density.

Fig. 4. Efficiencies of force transmission of single muscle fiber at different active force generation, (a) Comparison between muscle fibers with control and sparse proteins; (b) Comparison between muscle fibers with control and compliant proteins. The “total force generated in myofiber” refers to the active force generated by myofibers, i.e., F_a defined in Fig. 2.

Fig. 5.

Comparison of force distributions in proteins between muscle fibers with control and sparse densities of transmembrane proteins. The **horizontal** coordinates are normalized by half of the length of myofiber, L . The **vertical** coordinates are protein forces normalized by 75% of maximum force that proteins can support by definition.

Fig. 6.

Comparison of shear stress distribution in myofiber between fibers with control and sparse proteins. Each data point is calculated as an average shear stress of the two elements connecting to the transmembrane protein. All the stresses are normalized by the maximum shear stress in the single muscle fiber with sparse proteins throughout the interface.

Fig. 7.

Effects of location-specific density of the transmembrane proteins on the distribution of forces in the transmembrane proteins. The **horizontal** coordinates are normalized by half of the length of myofiber, L ; and the **vertical** coordinates are normalized by 75% of maximum force that proteins can support by definition. “Sparse middle” and “sparse end” represent fewer proteins in the middle only and at the end only, respectively.

Fig. 8.

Efficiencies of force transmission of single muscle fiber at different active force generation level with different distributions of densities of the transmembrane proteins. “Sparse middle” and “sparse end” represent fewer proteins in the middle only and fewer proteins at the end only, respectively. The “total force generated in myofiber” refers to the active force generated by myofibers (F_a).