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Short communication

In vivo simultaneous evaluations of sarcomere imaging and muscle fiber tension

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ABSTRACT

Muscle fiber tension and sarcomere length play critical roles in regulating muscle functions and adaptations under pathological conditions. However, methods are lacking to quantify these two variables simultaneously *in vivo*. A novel force microscope was developed with the unique capabilities of estimating muscle fiber tension and acquiring sarcomere images simultaneously *in vivo*. The force microscope consisting of a custom microscopic imaging system and a force sensor was used to quantify *in vivo* sarcomere length, muscle fiber tension and stress of the tibialis cranialis muscle at plantar-flexed and dorsi-flexed positions from 11 rat hind limbs. Results showed that sarcomere images and fiber tension could be measured together *in vivo* with significantly higher muscle fiber tension and stress and longer sarcomere length at the plantar-flexed position when compared to their counterparts at the dorsi-flexed position. The fiber tension estimated using the force microscope had close agreement with the direct measurements of the fiber tension. The present force microscope with simultaneous characterizations of fiber tension and sarcomere imaging provides us a useful *in vivo* tool to investigate the roles of muscle tension in regulating sarcomere and muscle fiber functions under physiological and pathological conditions.

1. Introduction

Sarcomeres are the smallest functional units of muscle and can be modified in response to mechanical loads (Goldspink and Williams, 1990; Jaalouk and Lammerding, 2009). For example, alterations in sarcomere morphology have been observed following prolonged immobilization and stretching (Goldspink, 1985; Herring et al., 1984; Koh and Herzog, 1998). Theoretically, sarcomerogenesis (*i.e.* sarcomere assembly) is achieved *via* ordered pathways (Ehler and Gautel, 2008) and responds dynamically to mechanical stimulus in order to regulate the optimal operation range of the sarcomere length (Herring et al., 1984). Sarcomerogenesis may not be evident due to neurological impairments as Lieber and Friden (2002) found that sarcomeres of spastic muscles were significantly longer than the sarcomeres of non-spastic muscles in an *in vivo* experiment. However, little information is currently available regarding the longitudinal interactions between the sarcomeres and the mechanical stimulus imposing upon them, such as tension fluctuations as a result of neurological impairments. This gap in knowledge is possibly due to a lack of appropriate methodology to quantify these two critical variables *in vivo* simultaneously. The purpose of this paper was to present and validate a force microscope with unique capabilities of estimating muscle fiber tension and acquiring sarcomere morphology simultaneously *in vivo*.

2. Methods

2.1. Experimental setup

Fig. 1 illustrates our developed *in vivo* force microscope and the way to estimate *in vivo* fiber tension (Zhang et al., 2011). To estimate the axial tension (F_x) of a fiber

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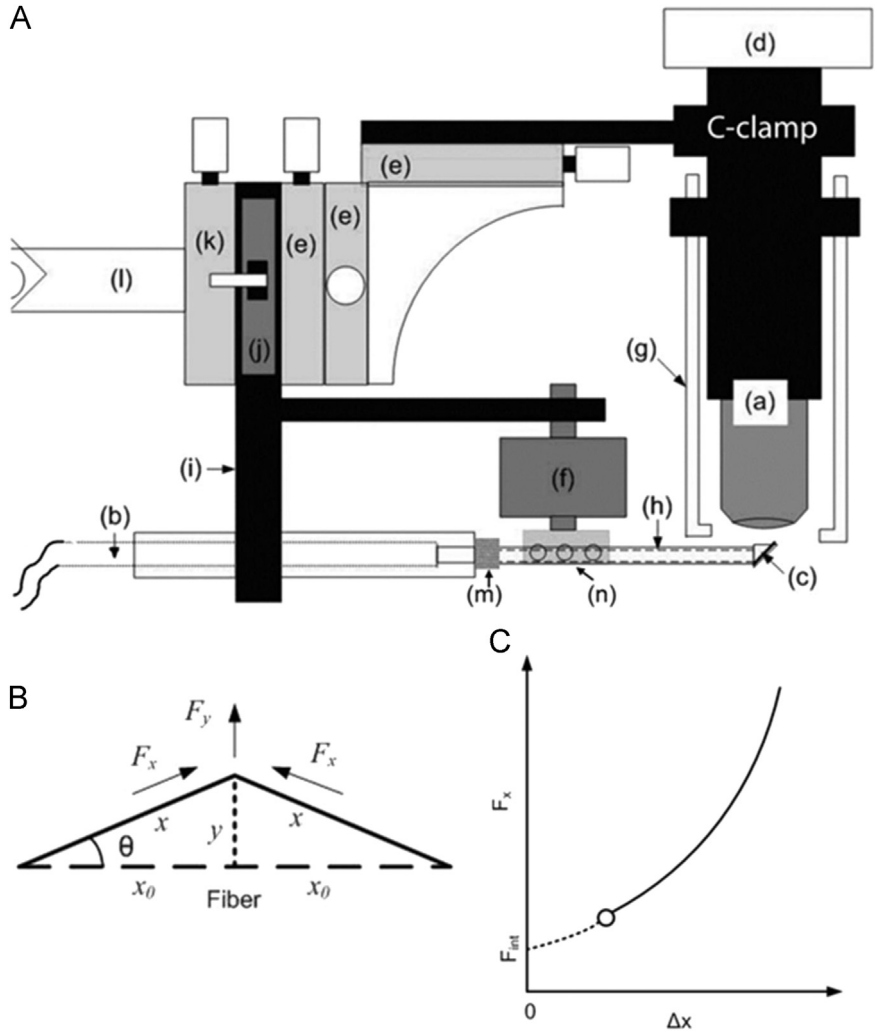

Fig. 1. (A) The *in vivo* force microscope system. An inverted microscope consisted of an 80 × objective and tube lens (a) and right angle prism (c) for changing light path as well as an XYZ position stages (e) for adjusting the focus and field of view. Light from the light source goes through the flexible fiber optic guide (b) and travels through the optical fiber inside the mental tube (h). A cover (m) is placed between metal tube (h) and light source (b). A CCD camera (d) is used to record images of the specimen. A load cell (f) is used to measure the lifting force applied to the fibers by the metal tube (h). The metal tube is secured to the load cell by multiple screws (n). A pair of fiber holders (g) is designed to hold the fiber bundle in place during lifting and measurement. The microscope and force sensing parts are mounted on a vertical post (i). A linear potentiometer (j) is used to measure the distance of lifting generated by the lifting stage (k) mounted to the arm of the boom stand (l). (B) shows the relationship between the axial tension (F_x) and the lifting force (F_y) with x representing half of the fiber bundle length, x_0 half of the initial fiber bundle length, y the lifting distance, and θ the lifting angle of the fiber bundle. (C) shows the axial force (F_x) curve-fitted as a function of the fiber elongation Δx.

bundle, the bundle was lifted vertically through the tip of the stainless tube (Fig. 1A (h)). The corresponding lifting force (F_y) and the vertical displacement (y) were recorded. F_x could be determined using trigonometry and force equilibrium calculations (Fig. 1B) as

$$F_x = \frac{F_y}{2\ \sin(\theta)} \tag{1}$$

$$\theta = \tan^{-1}\left(\frac{y}{x_0}\right) \tag{2}$$

where θ was the lifting angle and x_0 was half of the initial length of the fiber bundle.

Considering that F_x determined at small lifting angles involved relatively large error, especially at $\theta = 0°$ where F_x became undefined according to Eq. (1), F_x was only estimated at a lifting angle greater than 10°. F_x during a small lifting angle (*i.e.*, < 10°) can be estimated by fitting the calculated F_x as an exponential function (Eq. (4)) of the muscle fiber bundle elongation (Eq. (3)) with parameters C_1 and C_2 (Colomo et al., 1997). The initial axial tension (F_{int}) can be determined by extending the curve derived from Eq. (4) to the ordinate, with the intercept at $\Delta x = 0$ (Fig. 1C).

$$\Delta x = x - x_0 = \sqrt{x_0^2 + y^2} - x_0 \tag{3}$$

$$F_x = C_1 e^{C_2 \Delta x} \tag{4}$$

To validate the force microscope, we first investigated the biomechanical properties (*in vivo* passive length–tension relationships and derived tension and stress) of the tibialis cranialis (TC) fibers of rats at 45° plantar-flexion and 45° dorsi-flexion in a random order using the developed system followed by corroborating the estimated axial tension of dissected bundles using the direct measurement setup (Fig. 2A). The direct measurement setup consists of a miniature load cell (Force sensor X in Fig. 2A; M31, Honeywell Sensotec, Columbus, Ohio, USA) and a linear servomotor (Fig. 2A; M-230.10, Physik Instrumente, Karlsruhe, Germany) mounted on a positioning stage respectively for proper alignment.

2.2. *In vivo passive length–tension relationship, tension and stress*

Eleven small bundle (7.79 ± 2.39 mm in length and 1.7 ± 0.55 mm in width under the neutral ankle position) samples of the TC from male Sprague-Dawley rats (300–400 g) were isolated while the two ends still attached to the remaining muscle bulk. After placing the ankle at a desired position (45° plantar-flexion or 45° dorsi-flexion, in a random order), the initial length of the prepared fiber bundle was measured using an electronic caliper under a dissection microscope. The tube of the force microscope (Fig. 1A(h)) was then inserted underneath the isolated fiber bundle so that the bundle was right above the prism, (c) in Fig. 1A. This right-angle prism bended the light 90°, which illuminated the fiber bundle and through the lens ((a) in Fig. 1A), the fiber image was then formed on the CCD camera ((d) in Fig. 1A). When the fiber bundle was lifted, the force measurement and illumination