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Short communication

A novel muscle biopsy clamp yields accurate *in vivo* sarcomere length values

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ABSTRACT

The measurement of *in vivo* muscle sarcomere length facilitates the definition of *in vivo* muscle functional properties and comparison of muscle designs amongst functional muscle groups. *In vivo* sarcomere lengths are available for just a handful of human muscles, largely due to the technical challenges associated with their measurement. The purpose of this report was to develop and test a muscle biopsy clamp that can quickly and accurately measure *in vivo* muscle sarcomere length. To test the device, muscle biopsies ($n = 23$) were removed from the tibialis anterior muscles of New Zealand White rabbits immediately after sarcomere length measurements were made using laser diffraction. The muscle biopsy contained within the clamp was immediately fixed in Formalin for subsequent sarcomere length measurement. Comparisons of clamp-based and diffraction-based sarcomere lengths demonstrated excellent agreement between the two techniques, especially when the biopsy was obtained at relatively long lengths (above 2.6 μm). Given the intraoperative speed and simplicity of this technique and the relatively low-cost of the biopsy clamp, this method of measuring muscle sarcomere length should help investigators generate much-needed *in vivo* muscle structural and functional data.

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1. Introduction

Muscle architecture measurements provide quantitative estimates of muscle performance (Williams and Goldspink, 1978; Bodine et al., 1982; Powell et al., 1984). These values are critical input parameters for biomechanical modeling of the musculoskeletal system. However, architectural values only provide an estimate of the maximum force-generating potential (Powell et al., 1984), maximum shortening velocity (Bodine et al., 1982), or maximum excursion (Williams and Goldspink, 1978) of a muscle. Muscle physiological properties, specifically the length–tension and force–velocity relations, provide a more functional understanding of muscle performance. In fact, these characteristics can modulate muscle force by 100%. Therefore, accurate estimates of a muscle's *in vivo* functional properties are required.

To characterize muscle sarcomere length–tension properties, investigators have traditionally relied on microscopy to estimate *in vitro* sarcomere length on a very small muscle sample. In the 1930s it was discovered that coherent light, when passed through a muscle cell, diffracted in such a way that sarcomere lengths could be measured (Sandow, 1936a, b). Since that time, lasers have been used as the source of coherent light to measure sarcomere lengths *in situ* (Cleworth and Edman, 1972). This technique, which was fully developed in the 1980s and 1990s (Yeh et al., 1980; Lieber et al., 1984, 1994; Lieber and Boakes, 1988), has been used to characterize the *in vivo* sarcomere length-joint angle relations of muscles in a variety of systems including humans (Lieber and Boakes, 1988; Lieber and Brown, 1993; Lieber et al., 1994). The results of these studies have yielded valuable information to the modeling community regarding the *in vivo* length–tension behavior of muscle. However, *in vivo* laser diffraction can be a difficult, time consuming, and expensive endeavor that has only been performed in selected muscles. These muscles must be fairly superficial, exposed in a bloodless field, and have relatively simple architecture.

To address the critical need for understanding the *in vivo* sarcomere length–joint angle relations of many muscles that are anatomically deep and vulnerable to obscuring by blood, an alternative to laser diffraction has been developed. Therefore, the purpose of this study was to develop and test a device to measure sarcomere lengths *in vivo*, where laser diffraction is not possible. Here, we present a simple muscle biopsy clamp that quickly and accurately samples *in vivo* muscle sarcomere lengths.

2. Methods

Fourteen centimeter long stainless steel hemostat clamps (Model 3-113-14, Sabri Group, Pompano Beach, FL, USA) were modified by attaching custom 1 cm wide serrated jaws to their ends (Fig. 1). The jaws were machined from stainless

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steel (316) blocks using wire electrical discharge machining (EDM) to achieve very tight tolerances ($\pm$0.0005 cm) between mating jaw serrations (Fig. 1, inset). After the jaws were machined, they were welded to the hemostat jaws using tungsten inter gas (TIG) and polished. This machining process allowed the jaws to be sterilized using standard autoclaving and provided sufficient clamping pressure to prevent slippage of muscle fibers between the jaws.

To validate sarcomere lengths obtained using the clamp-based method, biopsies ($n = 23$) of the tibialis anterior muscles of New Zealand White rabbits ($n = 19$) were sampled. Animals were induced and maintained under gas anesthesia (isoflurane 2%). The Veterans Administration Institutional Animal Care and Use Committee approved all procedures.

The skin and fascia covering the anterior compartment was incised and reflected to expose the muscle. Micro-Adson forceps (Model 11018-12, Fine Science Tools, Foster City, CA, USA) and Metzenbaum scissors (Model 14018-13, Fine Science Tools, Foster City, CA, USA) were used to isolate small tibialis anterior fiber bundles approximately 2 cm in length. An intraoperative laser diffraction device (Lieber et al., 1994) was then placed deep to the isolated bundle, taking care to maintain the *in situ* trajectory of the muscle fibers (Fig. 2A). The foot was then placed in a position between full dorsiflexion and maximum plantar flexion to capture the full range of passive sarcomere lengths available in the tibialis anterior muscle. Once a foot position was chosen, the laser was inserted beneath the bundle and the distance between the +1 to −1 or +2 to −2 diffraction bands was measured and converted to sarcomere length as previously described (Lieber et al., 1994). This value was used as the "gold standard" sarcomere length value as it has been shown to represent sarcomere lengths throughout a passive muscle (Takahashi et al., 2007). Maintaining the foot position, the laser tip was then removed from the muscle and the biopsy clamp was placed around the fiber bundle in the same location as the laser tip. The jaws were closed (Fig. 2B) and the fiber bundle was cut proximally and distally to the clamp, removed with the muscle fibers, and submerged in Formalin. In some cases ($n = 4$) two biopsies were taken from the same muscles at different joint positions. After 24 h of fixation, the fiber bundle was removed from the assembly and placed on a glass slide for a second laser diffraction measurement (Lieber and Blevins, 1989).

In a subset of biopsies ($n = 16$), sarcomere lengths were measured *in situ*, after clamping alone, and after clamping with subsequent fixation. This allowed us to determine if the source of the sarcomere length measurement error was related to the fixation process or the clamping process.

The intraclass correlation coefficient (equation$_{2,1}$), simple linear regression, and average percent error was used to validate the technique. Data are presented as mean$\pm$SE, α was set at 0.05, and data were analyzed using SPSS (version 16.0, SPSS, Chicago, IL).

3. Results and discussion

Analysis of data from all 23 biopsies revealed that there was excellent agreement ($ICC_{2,1} = 0.929$, $r^2 = 0.77$, average error = 5.3%) between diffraction-based and biopsy-based sarcomere lengths (Fig. 3A). However, it was apparent that biopsy-based sarcomere length error was larger when *in vivo* sarcomere length was less than 2.6 μm (Fig. 3B). When sarcomere lengths were restricted to values greater than 2.6 μm, the agreement between techniques was even better ($ICC_{2,1} = 0.972$, $r^2 = 0.92$, average error = 2.5%). This is an interesting finding in light of the fact that rabbit muscle fibers would be expected to generate significant passive tension at lengths greater than 2.6 μm. This suggests that the biopsy clamp may apply a slight longitudinal tension to the muscle fibers during the clamping process when they are on slack. However, when fibers are under moderate passive tension (i.e. at sarcomere lengths greater than 2.6 μm), the fibers resist the small longitudinal tension created by clamping which allows the clamp-based method to yield more accurate sarcomere lengths.

To characterize the source of the clamp-based measurement error, some biopsies were measured *in situ*, immediately after clamping, and after clamping and fixation. These data demonstrated that fixation did not introduce systematic sarcomere length error (Fig. 3C), rather, the error was introduced by the clamping process itself at short sarcomere lengths (Fig. 3C).

4. Summary

In vivo sarcomere length can be estimated accurately using a muscle biopsy clamp that preserves *in vivo* sarcomere lengths. Using a simple goniometer, joint angle can be accurately

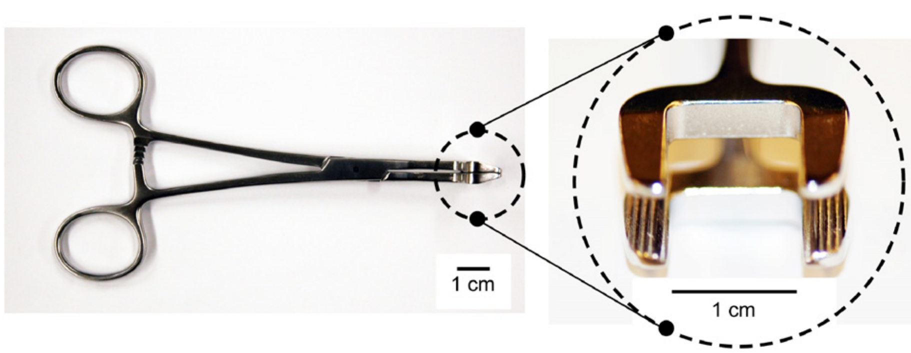

Fig. 1. Photographs of the muscle biopsy clamp device. A Side view of the modified Kelly clamp with jaws welded to their ends is shown along with a close-up view of the clamp jaws revealing precision-machined serrations.

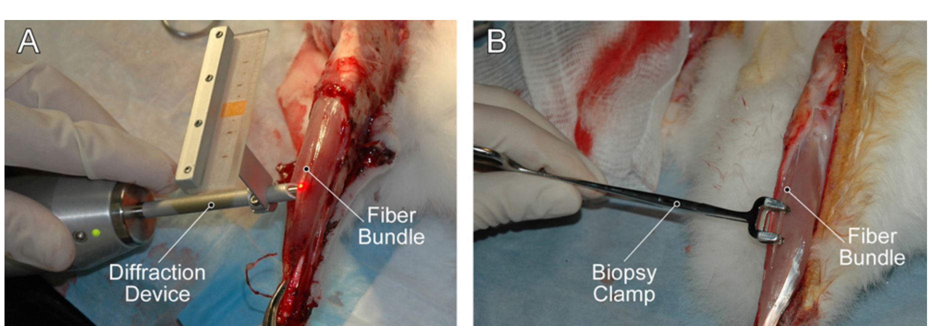

Fig. 2. Photograph of sarcomere length measurements on a tibialis anterior muscle fascicle. (A) Laser diffraction device beneath fascicle to measure sarcomere length. (B) Muscle biopsy clamp in place to harvest the fascicle.

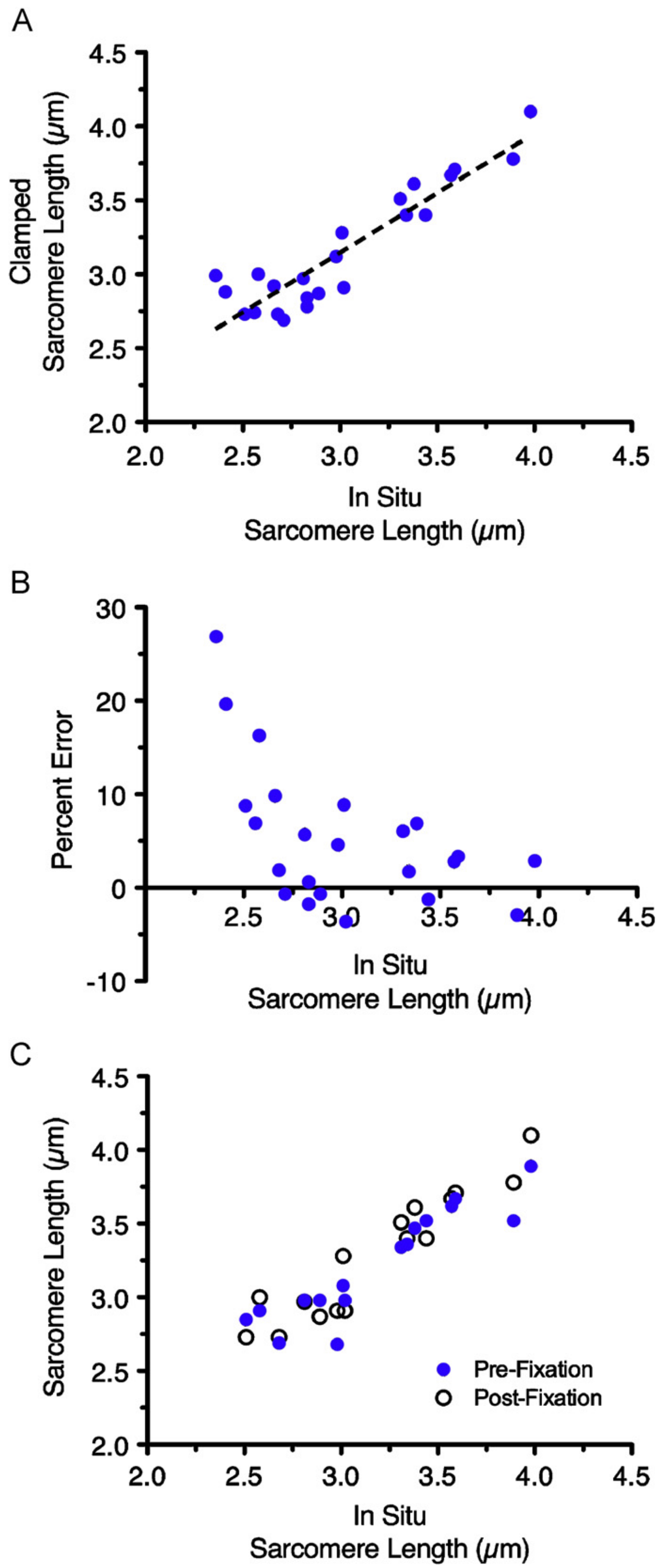

Fig. 3. Comparison of diffraction-based and clamp-based sarcomere lengths. (A) Scatter plot of diffraction-based sarcomere lengths (*x*-axis) vs. clamp-based sarcomere lengths (*y*-axis) demonstrating excellent agreement between the two techniques. (B) Scatter plot of the magnitude of clamp-based error (*y*-axis) vs. diffraction-based sarcomere length (*x*-axis). Note that acceptable error (~10%) can be obtained above 2.6 μm. (C) Sarcomere length data obtained in the clamp before and after fixation (*y*-axis) demonstrates that fixation is not the primary source of error in this method.

measured (Gogia et al., 1987), providing a reference position for sarcomere length measurements. Together, these tools allow sarcomere length–joint angle relationships to be defined for a greater variety of muscles (i.e. non superficial) than could be studied by laser diffraction. Although the described technique still used laser diffraction to obtain sarcomere length of fixed tissue, light microscopy could also easily be used to measure sarcomere lengths after fixation (Huxley and Peachy, 1961).

When using these data as input variables for musculoskeletal models it is important to consider that the biopsy allows average sarcomere length to be measured in a single bundle of muscle fibers. Although it has been demonstrated that a single sarcomere length value represents whole muscle average sarcomere lengths in passive muscle (Takahashi et al., 2007), it has been suggested that sarcomere lengths may vary regionally within active muscles due to extramuscular myofascial force transmission (Maas et al., 2003; Yucesoy et al., 2006, 2007), heterogeneous tendon strains (Lieber et al., 1992), or regional moment arm differences in muscles with broad insertions (Blemker and Delp, 2005; Blemker et al., 2005). Additionally, it has been suggested (Herzog and ter Keurs, 1988) that sarcomere length–tension relations do not precisely scale to whole muscle length–tension relations in all cases. Therefore, biopsy-based sarcomere length–joint angle data only yield an estimate of whole muscle length–tension behavior. Accurate modeled muscle forces may require additional information to achieve the level of accuracy required for an individual experiment.

In the development stage of this clamp, we attempted to manufacture jaw widths of 0.5, 1.0, and 1.5 cm. In terms of fabrication, 0.5 cm was too small to be precisely machined with wire EDM and 1.5 cm yielded an unnecessarily large sample. A jaw width of 1.0 cm provided us with a large enough sample to obtain accurate sarcomere lengths and harvest a relatively small length of a muscle fascicle. Although we have not attempted to measure the active force-generating properties of these biopsies we would expect them to be compromised, as the fiber membranes have been disturbed at the cut ends. Since the amount of tissue removed is less than a percent of the whole muscle, functional properties of the native tissue would not be measurably compromised.

There are several technical advantages to this technique over laser diffraction. First, laser diffraction is vulnerable to light scattering by blood and connective tissue in the surgical field. In these cases, a visible diffraction pattern simply cannot be obtained. Second, laser diffraction is limited to relatively shallow and wide surgical exposures. In cases where the muscle of interest is deep (e.g. spinal musculature and hip musculature) or the surgical field is narrow (e.g. minimally invasive surgical techniques), the laser tip cannot be placed deep to a muscle fiber bundle without elevating the bundle, which yields artificially long sarcomere lengths. Third, hand-held intraoperative lasers are expensive and require special sterilization (i.e. gas or gas-plasma) to preserve the electronic circuitry. Finally, intraoperative laser diffraction requires significant practice to achieve the skill level required to obtain accurate measurements in the very narrow time window provided during surgery.

There are also several technical limitations to the biopsy clamp technique. First, like laser diffraction, it is important to sample sarcomere lengths in passive muscle at relatively long lengths and to maintain the muscle sample in its *in situ* configuration. A common mistake is to sample passive sarcomere lengths at relatively short lengths, where the entire muscle tendon unit is on slack. In this case, one is essentially measuring slack sarcomere length as there no restoring forces at the level of the sarcomere below this point. The effect would be overestimation of sarcomere length at joint angles corresponding to small muscle-tendon unit lengths. Another common mistake is to pull the muscle fascicle up and out of the muscle belly when obtaining the biopsy or making the laser diffraction measurement which results in sarcomere length overestimation. Second, the clamp requires removal of the muscle sample which may not be acceptable. Third, this technique requires multiple biopsies to sample the sarcomere length–joint angle relationship at a variety of joint angles. In this situation, laser diffraction is a superior technique. Finally, the clamping method can only be used to make measurements in passive muscle while laser diffraction can, in principle, provide active and/or dynamic sarcomere length values. As with most areas of science, the choice of tool will depend on the specific question being addressed.

Conflict of interest statement

At the time of submission, the authors do not have financial or personal conflicts of interest that could influence (bias) the work presented in this manuscript.

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