A Microbiopsy Method for Immunohistological and Morphological Analysis: A Pilot Study

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ABSTRACT


Introduction: The fine aspiration microbiopsy is a relatively new biopsy technique, which allows muscle physiologists to sample skeletal muscle less invasively. However, the small sample size obtained is often deemed insufficient for certain analyses. The aim of the current study was to develop procedures for muscle fiber morphology and immunohistological analysis from a microbiopsy technique.

Methods: Microbiopsies of the vastus lateralis were taken with a 14-gauge microbiopsy needle from four healthy men on two separate occasions. The tissue was oriented in a cryomold, embedded in Tissue-Tek® then frozen in liquid nitrogen cooled isopentane. The muscle sections were stained with hematoxylin and eosin, laminin, MHCI, MHCIIa, and Pax7 for fiber number, mean fiber area, muscle fiber typing, and satellite cell observation.

Results: The mean ± SD (range) microbiopsy sample weight was 18.3 ± 2.9 mg (14–22 mg). The mean fiber number within the microbiopsy specimens was 150.4 ± 120.6 (64–366). All viable fibers were measured in each sample, and the mean fiber area was 4385.1 ± 1265.8 μm² (977.0–10,132.93 μm²). There was no significant time difference (P = 0.69) in mean fiber area.

Discussion: Results suggest the potential use of a “minimally invasive” muscle biopsy technique for immunohistological and morphological analysis. This could provide clinicians and investigators additional data in future research. Further investigations are needed to determine the usefulness and potential limiting factors of this technique. Key Words: FINE NEEDLE ASPIRATION, HISTOLOGY, SKELETAL MUSCLE, BIOPSY

Since its introduction in 1962, the Bergström needle muscle biopsy technique has long been perceived as the gold standard in muscle tissue sampling (1). This procedure has subsequently provided a wealth of information for researchers and clinicians alike, thus establishing itself as an indispensable tool in the morphological, biochemical, and pathological analysis of human muscle tissue (2,4). Despite its value, the Bergström biopsy technique is moderately invasive, requiring an incision through the skin and fascia ranging from 5 to 10 mm (2). Consequently, this technique may not be suitable for repeated sampling research designs.

More recently, the microbiopsy (or fine needle aspiration) has been implemented as a minimally invasive technique using a small gauge needle for tissue sampling without the need for an incision (5,10). Microbiopsy applications have been limited to histochemical and pathological analyses with little recognized value to histology because of perceived inconsistencies with sample weight and morphological integrity (7). To the authors’ knowledge, only one study has investigated the potential of a microbiopsy for histological analysis (7); however, these authors did not attempt immunohistochemical staining and analysis. Thus, the aim of the current pilot study was twofold. First, this study aimed to determine whether a sufficient number of muscle fibers can be obtained and analyzed from a microbiopsy, and second, it aimed to ascertain whether immunohistochemical analysis is procedurally feasible from a small amount of muscle tissue obtained from a microbiopsy technique.
MATERIALS AND METHODS

Four healthy men (26.8 ± 5.4 yr, 175.9 ± 4.3 cm, 91.9 ± 5.3 kg) volunteered to participate. Eight ipsilateral percutaneous microbiopsies were taken from the vastus lateralis with a 14-gauge microbiopsy needle on two separate occasions separated by 1 wk. All participants gave their informed consent, and the research study was approved by the university institutional review board.

The sampling location for the biopsy was determined by the point of intersection between the vastus lateralis and one-third of the straight-line distance from the greater trochanter and the lateral epicondyle of the femur. To confirm the site of the muscle belly for placement of the biopsy needle, ultrasonography was performed using a linear probe (LOGIQ P5; General Electric, Wauwatosa, WI) to optimize spatial resolution. The probe was coated with a water-based conduction gel (Aquasonic 100 ultrasound transmission gel; Parker Laboratories, Inc., Fairfield, NJ) and positioned on the surface of the skin to provide acoustic contact without depressing the dermal layer (14). Subsequently, the area over the outside vastus lateralis muscle was cleaned and disinfected with a topical antiseptic (Betadine; Stamford, CT). A 1.5-mL 1% lidocaine anesthetic was injected into and under the skin to reduce the discomfort of the biopsy. After the area was completely numbed, a small puncture to the skin was made using an 18-gauge needle and then a coaxial biopsy cannula was inserted (cat no. MCXS1410AX; Argon Medical, Athens, TX) in the vastus lateralis perpendicular to the muscle until the fascia was pierced using sterile technique (3). After placement of the biopsy needle (cat no. 765014100; Argon Medical, Athens, TX) into the automatic biopsy instrument (cat no. 7675, Pro-Mag™; Argon Medical, Athens, TX), the biopsy needle was then inserted through the cannula and into the muscle (Fig. 1). Muscle samples were obtained by the activation of a trigger button on the biopsy gun, which unloaded the spring and engaged the needle to collect a tissue sample. The biopsy needle was then slid out of the insertion cannula while the cannula was maintained in place, thus avoiding repeated skin punctures. Subsequent biopsies were obtained 2 cm proximal or distal from the first biopsy location.

Samples were removed from the biopsy needle with a sterile needle or scalpel and promptly weighed. The tissue was then placed and oriented in a cryomold (cat no. 22-363-553; Fisherbrand) to be embedded. First, a small amount of Tissue-Tek® was applied over the sample then frozen in liquid nitrogen cooled isopentane. The mold was then promptly removed from the isopentane, and the frozen sample was covered in Tissue-Tek® to completely fill the cryomold then placed back into the isopentane for rapid freezing (Fig. 2). Frozen samples were then stored at −80°C until further cryosectioning. For cryosectioning, the entire length of the embedded tissue sample was frozen to a tissue “chuck” and transverse cryosections (8 μm thick) were prepared. Muscle sections were stained with hematoxylin and eosin or antilaminin (cat no. 2E8; Hybridoma Bank, Ames, IA). Samples were then counterstained with an appropriately conjugated fluorescent antibody (Alexa Flour 350®) then coverslipped with ProLong® Gold Antifade Mountant (cat no. P36934).

Myosin heavy chain and Pax 7 staining. A few remaining samples were stained for myosin heavy chain I (cat no. BA-D5; Hybridoma Bank) and II (cat no. SC-71,
Hybridoma Bank) composition and counterstained with appropriately conjugated secondary antibodies (Alexa Flour 350 for myosin heavy chain I and Alexa Flour 647 for II). In addition, some samples were stained for Pax 7 satellite cells, (PAX7, Hybridoma Bank), myosin heavy chain I (cat no. BA-D5; Hybridoma Bank) and antilaminin (cat no. 2E8; Hybridoma Bank, Ames, IA). These sections were then counterstained with appropriately conjugated secondary antibodies (Alexa Flour 350, Alexa Flour 488, and Alexa Flour 647).

Prepared samples were viewed with a light and fluorescent microscope (Nikon N-E; Nikon Instruments, Melville, NY) at 4×, 10×, and 40× resolution. Fiber number and mean fiber area were measured and determined once by Image J software (National Institute of Health, USA, version 1.45 s).

**Statistical analysis.** A Wilcoxon signed-rank test was run between the two samples obtained from each participant to determine whether there was any significant difference in mean fiber area between samples. In addition, reliability was evaluated with intraclass correlation coefficient (ICC) and SEM values.

**RESULTS**

A total of 1366 muscle fibers were analyzed from eight muscle biopsy samples obtained. The mean ± SD (range) micro biopsy sample weight was 18.3 ± 2.9 mg (14–22 mg). Fiber number within the micro biopsy specimens was 150.4 ± 120.6 (64–366). The test–retest reliability for fiber number was ICC = 0.34 (SEM, 198.7 μm²). The muscle fibers were measured in each sample, and the mean fiber area was 4385.1 ± 1265.8 μm² (977.0–10,132.93 μm²). A Wilcoxon signed-rank test revealed no significant difference (P = 0.69) in mean fiber area between the two sampling time points (7 d) from each participant. The test–retest reliability for mean fiber area was ICC = 0.92 (SEM, 522.3 μm²). Because a complete set slides for satellite cells and fiber typing was not possible, there was no statistical analysis performed; however, images are presented in Figure 3.

**DISCUSSION**

Because skeletal muscle tissue has the potential to provide an abundance of data for researchers and clinicians, the purpose of this study was to determine whether a less invasive biopsy providing less tissue had the capacity for meaningful histological analysis. With a pain level similar to a blood draw (13), the fine needle biopsy has been widely implemented and validated in different applications such as gene expression (3), cell signaling (6), and enzymatic activity (7). In summary, our findings confirm those of Hayot et al. (7) that a skeletal muscle biopsy has the potential to provide sufficient number of muscle fibers for histological analysis. However, Hayot et al. (7) had the specific aim of validating this biopsy technique to the traditional Bergström needle technique in analyzing citrate synthase, phosphofructokinase activity, and myosin heavy chain composition whereas the purpose of the present investigation was to determine whether a small tissue sample obtained from a biopsy is viable for histological analysis. Furthermore,
to the authors' knowledge, this is the first study to investigate immunohistological staining from a microbiopsy.

Strict criteria of fiber sample size required to provide meaningful analysis of a human skeletal muscle biopsy have not been set in the literature. In one case, as few as 10 fibers have been used to determine fiber size (12). More recently, McCall et al. (9) determined that 50 muscle fibers were sufficient to provide an accurate depiction of the entire biopsy, with strong correlations to the means of the 100-fiber measurement. In accordance with these findings, it has also been shown that only 50 muscle fibers are sufficient for assessment of myonuclear number. However, to reliably estimate satellite cell number, it seems at least 50 Type I and 75 Type II fibers are needed for reliable analysis because of the relatively low number of satellite cells found in a given muscle sample (8). Nevertheless, it is promising that analysis of our mean fiber numbers and fiber typing could be reliably representative of a much larger sample.

It is important to note that there are several inherent limitations, which come with the microbiopsy technique. Although we were able to attain good reliability regarding muscle fiber area comparable with that in a previous study using the Bergström technique (11), such reliability was not obtained with fiber number. This may put considerable limitations on the type of analyses that can be done. Thus, for analyses that require a larger number of fibers (satellite cells, pericytes), the Bergström technique would be likely necessary because it provides a larger, more consistent sample size. Likewise, it is possible that muscle fiber typing (% Type I, IIa, and IIb) may provide useful information but could present significant variation between sampling time points.

This pilot study indicates the possible use of microbiopsy technique for examining skeletal muscle fibers through routine hematoxylin and eosin staining as well as reporting the potential for more sophisticated immunofluorescent staining techniques. However, more research with a larger sample size is needed to confirm our results.

The authors declare no conflict of interest. There is no funding to disclose.

The results of the present study do not constitute endorsement by the American College of Sports Medicine.

REFERENCES


