Quantitative assessment of muscle damage in the mdx mouse model of Duchenne muscular dystrophy using polarization-sensitive optical coherence tomography

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Duchenne muscular dystrophy (DMD) is a lethal, X-chromosome linked muscle wasting disorder affecting mainly boys that is caused by a defect in the gene that encodes for the structural subsarcolemmal protein dystrophin (54). Initial muscle damage in boys with DMD can progress to myofiber necrosis, because absence or impaired functioning of the dystrophin protein leads to increased susceptibility of dystrophic myofibers to contractile damage after exercise (36). In DMD, repeated cycles of myofiber necrosis over many years lead to replacement of muscle tissue with fibrous connective tissue and fat (30, 37), resulting in progressive loss of muscle mass and function and early death, often due to cardiac or respiratory failure (4, 12). The dystrophic mdx mutant mouse (C57BL/10ScSn/mdx) is a model for DMD that is widely used in preclinical research (15, 16, 50). Assessment of disease progression in the mouse typically involves death of the animal for tissue sampling and histological analysis to identify muscle necrosis (15). Such analysis is both very time-consuming and its invasive nature precludes the possibility of longitudinal assessment of disease progression over time in a single animal, increasing experimental uncertainty and cost.

In whole living animals, noninvasive imaging techniques such as magnetic resonance imaging (MRI) (2, 25, 26, 31, 43, 47), ultrasonography (11, 42, 46), or X-ray computed tomography (CT) (39, 40, 44, 53) have shown potential for imaging muscle necrosis in vivo, with the option of repeated measurements over time on the same subject. MRI is able to provide three-dimensional (3-D) imaging data from tissue volumes and discriminate between healthy and dystrophic muscle in canine (25, 26) and murine (2, 31, 43, 47) animal models. Use of MRI contrast agents enables quantitative assessment of the permeability of dystrophic myofibers and improves imaging contrast (2, 43). However, the imaging resolution of MRI is insufficient to visualize individual myofibers. Furthermore, the cost and long acquisition time of scans remain a challenge for employing MRI in either clinical imaging or preclinical studies. Ultrasonography, due to its lower cost and shorter acquisition time, is preferable for routine clinical use. The imaging resolution of ultrasonography, however, is also not capable of differentiating individual myofibers (11, 42, 46). CT is employed in clinical studies of myopathies, and a resolution of 40 to 50 μm can be reached with high-resolution micro-CT imaging of small animals (39, 53). With iodinated contrast agent, the imaging resolution of micro-CT can be as fine as 16 μm (40) under ideal conditions. However, the disadvantages of lower soft tissue contrast and very high radiation dosage remain impediments in micro-CT imaging (39). The challenge is to provide a minimally invasive imaging technique that can view a wide area of tissue with deep penetration, combined with high resolution (38), and ideally with the potential to be applied in situ in living animals.

Recently developed optical imaging techniques, such as optical coherence tomography (OCT), offer the potential for high-resolution, minimally invasive imaging methodologies (10, 13). OCT is a reflectance-mode optical imaging modality that uses low-coherence interferometry combined with point
beams by detecting depth-resolved backscattered near-infrared light (typical operating wavelength bands lie in the vicinity of 800 nm and 1,300 nm) in a way that is analogous to ultrasonic pulse-echo imaging (21). OCT imaging has been demonstrated to be capable of displaying the changes in myofibers due to necrosis in whole ex vivo murine muscles (23, 24). Although OCT has demonstrated an ability to display myofibers, it does not lend itself to automatic quantification of the proportion (volume fraction) of necrotic tissue within a muscle sample (23, 24).

Polarization-sensitive optical coherence tomography (PS-OCT) is an extension of OCT that measures the optical birefringence of tissue by analyzing the polarization of the backscattered light (19). Birefringence is an optical property of an anisotropic material whereby the propagation speed (and hence refractive index) of light traveling through the material depends on the polarization state of the light. In linear birefringence, which is the property of interest here, light polarized linearly parallel to the optic axis of the uniaxial, birefringent material experiences a different refractive index than light polarized linearly orthogonal to the optic axis. The difference between these two refractive indices is the amount of linear birefringence of the material (3). Healthy skeletal muscle tissue exhibits high linear birefringence (hereafter birefringence) arising from the highly organized anisotropic cellular structure and arrangement of myofibers. The detected birefringence is a maximum for light incident orthogonal to the fiber axis, which is the optic axis in this case, and is zero for coaxial illumination. Previous studies have shown that birefringence in normal skeletal muscle is due both to the anisotropic arrangement of subcellular structure within myofibers (18), and the anisotropic molecular structure of the filament molecules contained in the myofibers (6, 52). By contrast, damaged or necrotic muscle loses its anisotropic ultrastructure, and possesses correspondingly low birefringence (22). Although collagen and other filamentous, nonmyofibrillar proteins also exhibit birefringence (45), muscle birefringence is mostly a result of myofiber structures (6, 18). We therefore postulate that it is possible to use PS-OCT to identify regions of necrosis within skeletal muscle by calculating the birefringence across the muscle, and locating the regions of abnormally low birefringence. A previous study suggests that PS-OCT can detect the damage induced by exercise in dystrophic mdx mouse muscles by determining the decrease in birefringence. However, the study did not extend to visualization of the areas of necrosis, nor to quantification of the proportion of necrotic tissue within the mdx muscles (33).

In this paper we present a novel imaging and analysis technique that uses PS-OCT to quantify the proportion of necrotic tissue in fresh (unfixed) skeletal muscle tissue. The technique quantifies the level of birefringence in muscle tissue, generating an image that is indicative of the tissue structure. The technique is demonstrated on ex vivo skeletal muscles from both dystrophic mdx mice and wild-type C57BL/10ScSn mice. The resulting birefringence images are validated against collocated histological sections. Automated segmentation of the birefringence image additionally provides a measure of the proportion (volume fraction) of necrotic tissue within the imaging field of view (FoV). The proportion of muscle necrosis assessed by this technique is favorably compared against the proportion of muscle necrosis obtained from manual assessment of the histological sections.

**MATERIALS AND METHODS**

**Animal handling.** All mice were obtained from the Animal Resources Center, Murdoch, Western Australia. Experiments conformed to the guidelines of the National Health and Medical Research Council Code of Practice for the Care and Use of Animals for Scientific Purposes (2004) and the Animal Welfare Act of Western Australia (2002), and were approved by The University of Western Australia Animal Ethics Committee. Six mdx mice (C57BL/10ScSn<sup>mdx</sup>) (male, 9–10 wk old) were exercised on a horizontal treadmill for 30 min at a speed of 12 m/min to increase the amount of necrosis in their skeletal muscles (36). Immediately after exercise, five of the mdx mice were injected with 1% (wt/vol) Evans blue dye (EBD; Sigma, St. Louis, Missouri) in phosphate-buffered saline (PBS) (pH 7.5) by intraperitoneal injection at a dose of 100 μl per 10 g body wt (17, 41). The remaining mdx mouse was imaged without EBD injection as a control sample. EBD has been shown to be a useful marker for identifying the onset of muscle damage by binding to serum albumin and readily diffusing only into myofibers with a permeable (damaged) membrane (15). The damaged EBD-positive myofibers could be identified macroscopically by their dark blue staining. This in situ staining was used to guide the subsequent imaging. In addition, four wild-type normal mice (C57BL/10ScSn<sup>mdxless</sup>) (male, 8–10 wk old) were sampled and imaged without exercise or EBD injection to provide necrosis-negative control (healthy) muscle samples. The C57 mice were not exercised because it was well documented that even strenuous exercise does not induce necrosis in normal wild-type mouse muscles (36).

**Sample collection.** All mice were anesthetized using 2% (vol/vol) isoflurane (Bomac, Australia), N<sub>2</sub>O, and O<sub>2</sub> and then killed via cervical dislocation. This occurred in the mdx mice 24 h after completing the treadmill running exercise. Skeletal muscles from both the forelimbs and hindlimbs were excised and immediately placed in PBS on ice. Muscles were excised by severing muscle-tendon and muscle-bone attachments; subsequent PS-OCT imaging was performed away from muscle-tendon junctions and muscle-bone attachment points to avoid any effect of excision-induced muscle trauma on the PS-OCT images. Muscles were selected for PS-OCT imaging on the basis of observation of EBD accumulation, indicating leaky myofibers and an increased likelihood of a necrotic lesion. These muscles were preferentially dissected and scanned during the time period available. Scanning of the muscle samples began within 2 h of excision. A total of 11 muscles were imaged from six mdx mice (7 from five mdx mice injected with EBD; 4 from one mdx mouse that was not injected with EBD), and 15 muscles were imaged from four C57 wild-type mice (that were not injected with EBD), as shown in Table 1.

**PS-OCT imaging.** PS-OCT acquires images of tissue by illuminating a biological sample with a weakly focused beam of polarized, near-infrared light and detecting the polarization of light that is backscattered (reflected) from the sample (9, 19). The backscattered light from a range of depths is detected simultaneously by the system, generating an image that is indicative of the tissue structure. PS-OCT acquires images of tissue by illuminating a biological sample with a weakly focused beam of polarized, near-infrared light and detecting the polarization of light that is backscattered (reflected) from the sample (9, 19). The backscattered light from a range of depths is detected simultaneously by the system.

<table>
<thead>
<tr>
<th>Mouse type</th>
<th>Number of mice/muscles/scan</th>
<th>EBD injection</th>
<th>Exercised</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mdx mice (dystrophic)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necrosis-positive</td>
<td>5/7/11</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>EBD-negative control</td>
<td>1/4/4</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Wild-type mice (healthy)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necrosis-negative control</td>
<td>4/15/34</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>EBD, Evans blue dye</td>
<td></td>
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The myofibers are degenerated with fragmented sarcoplasm. The muscle structure is highly degraded compared with the PS-OCT FoV, so light of linear incident polarization runs along the muscle fibers (18), and is approximately constant over linear at 45° to the sample’s optic axis. The optic axis of muscle tissue retardation is maximized when the incident polarization is circular, or (the incident polarization), the dynamic range of the detected phase retardation. These experiments that use a single polarization state incident on the sample to conduct birefringence of the tissue. For PS-OCT systems (such as the one used in these experiments) that use a single polarization state incident on the sample (the incident polarization), the dynamic range of the detected phase retardation is maximized when the incident polarization is circular, or linear at 45° to the sample’s optic axis. The optic axis of muscle tissue runs along the muscle fibers (18), and is approximately constant over the PS-OCT FoV, so light of linear incident polarization ~45° to the optic axis was sufficient for this study. The incident polarization state was manually adjusted before each scan to achieve the maximum dynamic range of the detected phase retardation.

All samples were imaged using a fiber optics-based PS-OCT imaging system (PsoCT-1300; Thorlabs, NJ) as shown in Fig. 1. Each intact muscle sample was placed on a glass coverslip and imaged through the coverslip from below. This minimized imaging artifacts by flattening the imaging surface and by reducing the refractive index mismatch when the light enters the tissue (28). Samples were kept hydrated using PBS during imaging. Three-dimensional PS-OCT scans were acquired, each covering a 4 × 4 (lateral) × 3 mm (depth) volume, with a resolution of ~16 μm (5, 27). Typically, two to three adjacent scans were required to cover the majority of each muscle. For muscle samples from the EBD-injected mdx mice, the presence of the dye provided an indication of areas of muscle damage. This was used to guide imaging toward regions that included a boundary between damaged and undamaged areas of muscle. For muscle samples from the non-EBD-injected mdx mouse, regions for imaging were selected using a real-time display of the PS-OCT phase retardation signal. For muscle samples from the C57 wild-type mice, the regions for imaging were selected to cover the whole muscle.

Post PS-OCT imaging histology. Immediately after imaging, the muscle samples were carefully moved onto cork boards to preserve their orientation. The samples were fixed to the cork with tragacanth gum or pins to prevent any movement, before being preserved either by freezing in liquid nitrogen-cooled isopentane, or fixed in formaldehyde solution (Confix Clear pH 7.0, AB1020.1000C; Australian Biostain) for future histological analyses. This ensured as much as possible that the subsequent histological sections were obtained parallel to the plane of the PS-OCT birefringence images. Histologic preparation was carried out on a series of 8-μm-thick sections that were obtained on a cryomicrotome with 120-μm intervals between adjacent sections throughout the depth of tissue, and stained with hematoxylin and eosin (H&E). Histological sections were digitally photographed at 20× magnification (Scanscope XT; Aperio Technologies, Vista, CA). Muscle regions in H&E-stained histological sections were classified as either normal-appearing or necrotic, on the basis of visual inspection of the photographed sections. Examples of normal-appearing muscle histology and two stages of necrotic muscle histology are shown in Fig. 2.

Generation of parametric PS-OCT birefringence images and calculation of percentage necrosis. The method of generating parametric PS-OCT images of birefringence is described fully by Chin et al. (5), but is summarized here for completeness. Each voxel in the acquired 3-D PS-OCT data set was converted into a 4-element Stokes vector, \( \hat{S} = \left[ I, Q, U, V \right]^T \), representing the polarization state of light backscattered from that location in the tissue (1). A standard OCT image was formed using the \( I \) component of the Stokes vector, and because PS-OCT detects only the fully polarized component of the light backscattered from tissue, \( \tilde{S} = \left[ Q, U, V \right]^T \), the reduced Stokes vector giving a narrow one-dimensional measurement into the tissue along the path of the light beam. The signal at each individual depth is acquired through a process referred to as low-coherence interferometry (19, 21). A 3-D data volume of polarization and backscatter by depth is acquired by scanning the beam in two directions across the surface of the sample. The polarization state at each depth is compared that measured at the surface of the tissue, and the rate of change of polarization (phase retardation) with depth gives a measure of the birefringence of the tissue. For PS-OCT systems (such as the one used to conduct these experiments) that use a single polarization state incident on the sample (the incident polarization), the dynamic range of the detected phase retardation is maximized when the incident polarization is circular, or linear at 45° to the sample’s optic axis. The optic axis of muscle tissue runs along the muscle fibers (18), and is approximately constant over the PS-OCT FoV, so light of linear incident polarization ~45° to the optic axis was sufficient for this study. The incident polarization state was manually adjusted before each scan to achieve the maximum dynamic range of the detected phase retardation.

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The tissue sample, was calculated as deemed to indicate necrotic tissue. The relative areas of necrotic tissue regions, and birefringence values below this threshold were

\[ \text{H}/H_{9272} \]

yield the wrapped phase \((1, 34)\). The phase retardation with depth, \(r\), of the smoothed Stokes vectors with depth into the tissue can be extracted from the rate of change of the phase angle, or phase constant during imaging, so the birefringence of the tissue could

\[ \text{H}/H_{9254}/H_{9272} \]

be calculated by counting the number of pixels in the birefringence images that lay below or above the threshold value, respectively. The percentage of muscle necrosis in each PS-OCT data set was then automatically calculated as the ratio of the area of necrotic muscle to the total area of muscle tissue in that data set. These percentage values were subsequently compared with the corresponding percentage values, which were manually estimated from histological sections of the volume of tissue represented by the PS-OCT images.

**Manual estimation of the percentage of muscle necrosis from histological sections.** Reference estimates of the percentage of necrotic tissue were obtained by manually delineating necrotic areas in the regions of the histological sections corresponding to the regions of the PS-OCT scans of the \(mdx\) mouse muscles. Photographs of the H&E-stained histological sections were manually segmented into necrotic and normal-appearing regions to a granularity of individual myofibers. Because PS-OCT scans a volume of tissue, each scan corresponds to a stack of histological sections through the depth of the sample. For each stack of histological sections, the percentage volume of muscle necrosis was calculated on the basis of the first nine sections (8 \(\mu m\) thick, 120 \(\mu m\) apart) from the top of the sample to ensure that the stack covered the range measured by PS-OCT. For each histological section the region corresponding to the area within the PS-OCT imaging FOV was located by visual coregistration of the section with the PS-OCT birefringence image, the OCT images, and the preceding histological sections in the stack. The areas of necrotic and normal-appearing tissue were then marked and calculated by manual assessment.

A relative measure of the amount of necrotic tissue within the PS-OCT imaging volume was estimated from the histological sections as the sum of the area of necrotic tissue from the FOV of the first nine sections. Similarly, a relative measure of the amount of muscle tissue, both necrotic and normal appearing, within the imaging volume was estimated as the sum of the area of both necrotic and normal-appearing tissue from the FOV of the nine sections. The percentage of necrotic tissue within the imaging volume was then calculated as \((\text{summed necrosis area}/(\text{summed necrosis area} + \text{summed normal-appearing area}) \times 100\%\), and compared with the corresponding percentage value calculated from the PS-OCT birefringence image.

To assess interobserver variability in this manual scoring, a test set comprising 10 histological sections photographed at 20× magnification was blindly scored by two trained observers. The test set consisted of 10 randomly selected H&E-stained sections taken from 7 different muscles from 4 of the EBD-injected \(mdx\) mice. The interobserver variability was calculated as the mean absolute difference of the percentage necrosis as assessed by these two observers.

**RESULTS**

Three representative sets of images are shown in this section. Two of them (Figs. 3 and 4) are of dystrophic muscles that were taken from two exercised \(mdx\) mice, and display areas of necrosis. The third set of images (Fig. 5) is a control set from healthy muscle tissue from a C57 wild type mouse. Each set contains four colocated images—a photograph of the excised muscle, an H&E-stained histological section, an OCT image, and the calculated PS-OCT birefringence image showing corresponding features of interest.

Figure 3A shows a photograph of an EBD-stained, excised, \(mdx\) mouse triceps muscle. The necrotic region is visible by its darker blue staining relative to the rest of the muscle. The corresponding H&E histology is shown in Fig. 3B, with areas of necrosis identified by fragmented muscle fibers and the appearance of purple-stained, basophilic inflammatory cells (with examples marked by * symbols). Figure 3, C and D shows the colocated OCT image and the parametric birefringence image computed from the PS-OCT data acquired on the

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intact, fresh (prefixation) tissue. The OCT image shows striations corresponding to intact myofibers and an area of low (dark) signal in the region of necrosis, in agreement with previously published findings (23, 24). The parametric birefringence image demonstrates a clear distinction between the high birefringence of the region of undamaged tissue (blue and green) and the region of necrosis (red). The corresponding values for the birefringence extracted from the PS-OCT signal are indicated by the color bar to the right of Fig. 3D.

Figure 4 shows a large region of necrosis present in a triceps muscle from a different mdx mouse enclosed on either side by normal-appearing muscle tissue. There is a clear correspondence between the region marked by EBD (Fig. 4A), areas of inflammatory cells and degraded myofibers in the histology (Fig. 4B), and the region of low birefringence observed in the PS-OCT image (Fig. 4D). These regions are notably less well differentiated in the standard OCT image (Fig. 4C), with faint striations still visible in the area of necrosis.

Figure 5 shows colocated images of healthy muscle taken from a C57 wild-type mouse. The H&E histology (Fig. 5B) shows all myofibers to be intact and well organized. The OCT image (Fig. 5C) shows a generally consistent striation pattern over the entire imaging region. Similarly, the parametric birefringence image of Fig. 5D measured a high birefringence (healthy/normal-appearing muscle) across the entire imaging field (blue and green). Some localized artifacts of low birefringence are present, due primarily to hairs and surface dirt obstructing and distorting the imaging light beam. Such small particles proved difficult to remove from the surface of the fresh tissue without risking damage to the samples.

Figure 6 shows the comparison between the manually estimated (from H&E histology) and automatically calculated (from PS-OCT) percentage of necrotic tissue within corresponding regions of dystrophic mdx mouse muscle samples. This comparison involves the 11 scans (with matching histology) from 7 different muscle samples from 5 different EBD-injected mdx mice. Although histology covers the entire muscle, this comparison is calculated only for the areas of the muscle samples that lay within the 4 × 4 mm FoV of the PS-OCT scans. The mean difference between the percentage necrosis calculated from PS-OCT and from histology was 2%. The error bars indicate the calculated interobserver variability for manual scoring of the H&E histology: 4.1 ± 4.7%, mean ± SD, calculated from the 10 test sections.

**DISCUSSION**

We have presented a new technique for the automated quantification of the amount of necrosis in fresh (unfixed) muscle tissue on the basis of a method for calculating birefringence from 3-D PS-OCT scan volumes. Visual assessment of the extent of necrosis may be made by inspection of the generated parametric birefringence images, and automated quantification of the proportion of necrotic tissue may be
performed by thresholding of the birefringence values. We suggest that this technique has the potential to complement current histological and imaging methods.

The optical birefringence of muscle tissue is primarily a result of the anisotropic structural organization of the myofibrils, and not of their genetic encoding (6, 18). For this reason, healthy muscle tissue, regenerated muscle tissue, and regions of normal-appearing tissue in dystrophic muscle exhibit comparable birefringence, allowing this technique to distinguish between normal-appearing and necrotic tissue in dystrophic muscle. Similarly, we noted that muscle tissue loses its birefringence at an early stage of the necrosis process, as seen for example, in the center left in Fig. 4. The histological section in Fig. 4B shows the myofibers in this region to be still relatively intact, although starting to fragment, but the birefringence in Fig. 4D is comparable to the regions in the center left of the image, which consist of highly fragmented myofibers.

We used systemic injection of EBD to guide imaging toward those muscle regions likely to contain necrosis. EBD stains necrotic regions dark blue due to the enhanced permeability of damaged myofibers. Note that whereas EBD was used to aid in the validation, it is not required in general usage of the technique. The imaging of a control, EBD-free mdx mouse was used to confirm that PS-OCT imaging is not affected by the presence or absence of EBD (data not shown). Treadmill exercise of mdx mice is also not required to perform PS-OCT imaging, but has been used here simply to increase the proportion of necrotic tissue assessed within these experiments.

Examination of individual histological sections provides a detailed 2-D assessment of the integrity of individual myofibers; however, the parametric birefringence images provide a more concise representation of the amount of necrosis within the 3-D volume. Differences in the estimated percentage (~2%) of necrotic tissue between an observer manually scoring H&E-stained histological sections and the proposed automated method were found to be comparable to the interobserver variability (4.1 ± 4.7%, mean ± SD). In general, the parametric birefringence images show good visual correspondence to the H&E-stained sections. However, we note small discrepancies between the parametric birefringence images and specific H&E-stained histological sections. This is due in part to the histological sections being 8 μm thick, whereas the birefringence image aggregates information over a 500-μm-thick volume of the tissue. For example, the band of low birefringence to the left of the birefringence image in Fig. 4D is not visible in the corresponding histologic image, Fig. 3C; however, an examination of subsequent and preceding histological sections reveals a region of fragmentary myofibers corresponding to this band of low birefringence. Additionally, tissue is subject to shrinkage and deformation during freezing and histological staining (20, 48), and this has the potential to affect both the location and relative size of areas of necrosis and normal-appearing tissue. It is also extremely difficult in practice to obtain a histological section in precisely the same orientation as the plane of the birefringence image, and this could further increase the discrepancy between features visible in the histological sections and in the birefringence images. We have conservatively attributed features visible in the PS-OCT
birefringence images, but not in the histological sections, to imaging artifacts; for example, the band of higher birefringence to the right in Fig. 3D, or the small regions of low birefringence in Fig. 5D. However, such imaging artifacts make only a small contribution to the estimates of the proportion of necrotic tissue, accounting for the good correspondence between the manual and automated estimates of the percentage volume of muscle necrosis.

Furthermore, we note that the measured birefringence of the muscle is dependent upon the orientation of the myofibers with respect to the optical beam. The maximum birefringence will be measured when the PS-OCT beam is oriented perpendicular to the longitudinal axis of the myofibers. In this case, the generated birefringence image will correspond to a histological section that cuts longitudinally across the myofibers. When the PS-OCT light beam is parallel to the longitudinal axis of the myofibers, no birefringence will be detected even in healthy or normal-appearing regions of the muscle sample. If the sample contains myofibers oriented in multiple directions, then PS-OCT imaging will tend to underestimate the birefringence of myofibers that are not oriented parallel to the plane of the birefringence image. However, the expected orientation of the myofibers is typically well understood from the morphology of the muscle, allowing optimization of the direction of PS-OCT scanning.

Muscle samples often exhibit regional variations in the severity of necrosis, as illustrated by the range of values for the proportion of necrotic tissue shown in Fig. 6 (i.e., for the same muscle from the same mouse, when acquired over a 4 × 4 mm subset of the muscle). This may cause difficulties in estimating the overall necrosis within dystrophic muscle when employing a PS-OCT system with a limited FoV (less than 10 × 10 mm). Within this study, scans were guided by the use of EBD as a marker of necrosis. In practice, multiple acquisitions across the muscle would be required during the assessment. However, recent improvements in OCT image acquisition rates (49) could potentially allow larger scans to be acquired in real time.

One challenge in extending the use of PS-OCT to an in vivo setting is its limited penetration depth in tissue, typically 1–2 mm. However, it has been shown that an OCT probe may be miniaturized and encased in a medical needle (8, 32, 35, 51). OCT needle probes capable of acquiring 3-D data volumes have been reported down to the size of a 30-gauge needle (outer diameter 0.31 mm) (29, 32), substantially smaller and less invasive than standard biopsy needles. Such an imaging probe could be used interstitially to image muscle structure in situ. A single needle scan, because it can image a greater area of muscle tissue, may be as informative as tissue removal, sectioning, and histological analysis. Insertion of a fine needle at several locations within one large muscle is potentially far more informative (and less invasive) than a single biopsy.

Conclusion. This paper presents a new technique that utilizes PS-OCT to image dystrophic skeletal muscle in a mouse model to estimate the proportion (volume fraction) of necrosis using a fully automated algorithm. PS-OCT detects the optical birefringence in skeletal muscle tissues with high birefringence values (>0.5 × 10⁻³) indicative of healthy skeletal muscle tissue and normal-appearing regions in dystrophic muscle tissue. Low birefringence values (<0.5 × 10⁻³) are indicative of necrotic (damaged) regions of myofibers (both early and later-stage damage indicated by inflammation) within dystrophic muscle. This report presents the first 3-D PS-OCT data volumes from both dystrophic and healthy mouse skeletal muscle samples, as well as the first quantitative color-coded parametric images differentiating normal-appearing from necrotic regions within skeletal muscle on the basis of calculated optical birefringence values. The percentage of necrotic tissue within the imaged locations on whole muscles was calculated and found to correspond well (mean difference of ~2%) to manual assessment of H&E-stained histological sections. This technique has the potential to provide a complementary, minimally invasive modality for analyzing and assessing the pathology of muscular dystrophy in situ.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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