MORPHOLOGICAL CHARACTERIZATION OF HEALTHY AND PATHOLOGICAL MUSCLES BY OPTICAL COHERENCE TOMOGRAPHY AND IMAGE ANALYSIS

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Introduction

Skeletal muscle comprises 40% of total body mass of mammals containing 50 to 75% of the body's protein [1], [2]. The most prevalent genetic muscle disease affecting children is Duchene muscular dystrophy (DMD) [1]. A new nondestructive technique called Optical Coherence Tomography (OCT) for morphological and mechanical characterization of tissues was used. OCT is a non-invasive optical method founded on the interferometry principle. In biological tissues, high scattering results in a loss of contrast, particularly for OCT images. Accordingly, several studies have used clearing agents (CA) to replace interstitial fluids [3],[4]. Accordingly, a morphological characterization of healthy and DMD rat muscles by OCT technique with Iodixanol (IOD) as CA was developed. By employing this 3D imaging method, we conducted a detailed description of structural differences of muscles. Additionally, as the tissue is preserved it could be used for upcoming mechanical tests.

Methods

Optical Coherence Tomography (OCT) imaging: An OCT system (Thorlabs OCT-TEL220C1) with a wavelength of 1.300nm, was used to assess the three-dimensional microstructure architecture of the sample. Muscles were thawed at room temperature in PBS. Then, muscles were fixed in a biaxial test machine by jaws and a preconditioning process was carried out. Thereafter, muscles were immersed in IOD for the clearing process and image acquisition was performed. The Weka tool in ImageJ was used to segmentate muscle fibers. **Muscle isolation:** Wistar rats, were used in this study. The protocol used in the present study complies with the principles of animal care and the French ethical rules of veterinary authorities (Council of Europe No. 123, Strasbourg 1985). Extensor digitorum longus (EDL) and soleus muscles were isolated from healthy and pathological rats for the analysis. **Statistical analysis:** The statistical analysis was performed using R software. A Mann-Whitney U test for a non-parametric population was performed to determine differences between control and DMD muscles fibers.

Results

Increase of homogeneous refractive index across the muscle and reduction of the scattering coefficient were induced by IOD. OCT images obtained after the filtering and the machine learning (Weka) are shown in Figure 1. Muscle fibers, in EDL control muscles, have a homogeneous shape and geometry. However, it can be observed that there are more round-shaped muscle fibers (red circles) in DMD muscles due to the symptoms and evolution of the pathology (Figure 1). Statistical differences of muscle fibers diameter and ECM area were analyzed. Results evidenced an increase in the number of fibers greater than 45 µm (Figure 2).

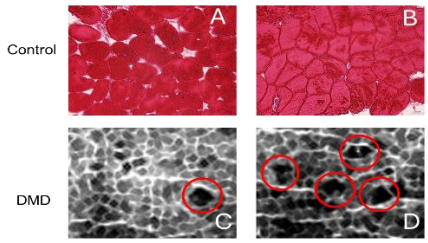

Figure 1: A,B) Histologies of soleus healthy muscles. C,D) OCT images of pathological EDL muscles (red circles).

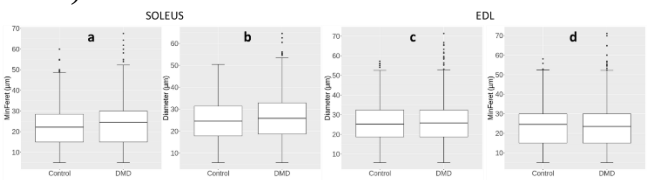

Figure 2: Minferet and diameter boxplots of healthy and pathological fiber for the soleus and EDL muscle.

Discussion

The application of new techniques like OCT enables to visualize and quantify the microscopic architecture of the healthy and pathological muscles without affecting the tissue, which gives the possibility to preserve the sample for further uses such as mechanical testing or molecular characterization. IOD as clearing agent enables us to develop a statistical analysis of muscle fibers morphology and extracellular matrix quantity for healthy or pathological muscles. The OCT, the image segmentation, and statistical analysis applied allowed us to characterize accurately pathological tissues like DMD muscles.

References

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