Transversal elasticity of TIEG1 KO muscle fibers probed by atomic force microscopy

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Introduction

Inhibition of TIEG1 gene provides changes within the musculoskeletal system [1-2] such as hyperplasia, hypertrophy and a metabolic glycolitic switch for slow and fast fibers [3]. Previously, mechanical tests have been performed on single muscle fibers using stretch and relaxation tests in the direction of the main fiber filaments. The functional properties of TIEG1 KO muscles showed mechanical changes compared to WT control muscles. Thus, the objective is to complete this characterization by applying transversal indentations to single fibers using a new experimental protocol adapted to atomic force microscopy (AFM).

Methods

Control (N=7) and TIEG1 KO (N=7) fibers were extracted from slow (soleus) and fast (EDL) muscles. To conduct transversal indentations, it is first necessary to immobilize the fiber during the indentation test. Thus, different fixations (glue, clips) and supports (glass, plexiglass) were tested. A drop of PBS was added on the surface of the fiber and the support was placed on an inverted optical microscope which is combined to the AFM. The AFM tip was scanned on three areas along each fiber (Fig1) by applying an indentation force (2 nN). Data were acquired in Quantitative Imaging mode and analyzed in-house with python Atomic Force software. The Young modulus was estimated for each force curve and displayed as colored pixels that reflect the magnitude of fiber stiffness (Fig2).

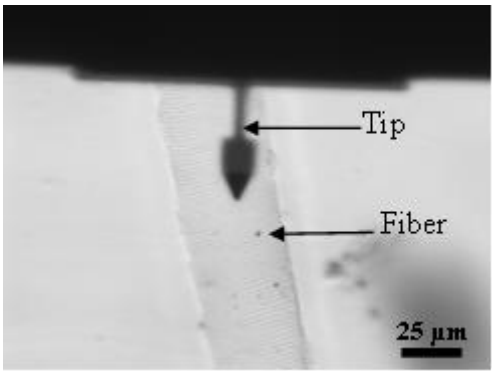

Fig.1: AFM tip scanning the muscle fiber

Results

No chemical agent was used to stabilize the fiber while using the plexiglass support. The elasticity maps revealed the fiber structure (sarcomers), demonstrating the capability of the experimental protocol and the post treatment of the data to measure elasticity of single fibers

with AFM. Young moduli were lower for TIEG1 KO fibers compared to WT control fibers as indicated by less intense color mapping for TIEG1 KO fibers.

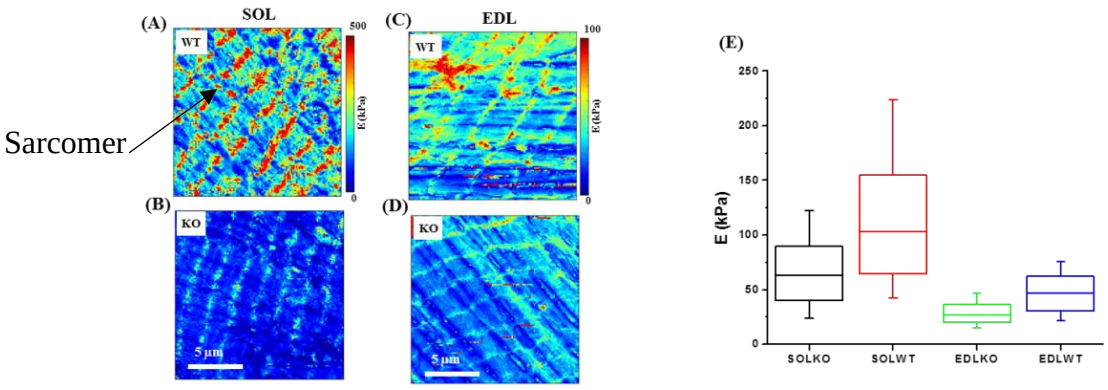

Fig.2: Elasticity maps (15x15µm) for slow (soleus) and fast (EDL) fibers in function of genotype. Box-whisker plots of elasticity obtained for the different fibers

Discussion

This is the first study using AFM for the elastic characterization of muscle fibers isolated from mice. Regardless of genotype, slow fibers showed higher elasticity than fast fibers. These results indicate a less organized structure (i. e. lower resistance under the tip) which is revealed by a less rigid muscle composition. These findings were magnified due to deletion of the TIEG1 gene. Further, these results are supported with the TEM acquisitions showing a disorganized structure for TIEG1 KO muscle.

Acknowledgements

This work was carried out and funded in the framework of the Labex MS2T. It was supported by the French Government, through the program "Investments for the future" managed by the National Agency for Research (Reference ANR-11-IDEX-0004-02).

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