MR Microscopy of Healthy and Dystrophic Muscle Fibers

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Introduction:

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder that results from mutation(s) in the dystrophin gene[1]. It is characterized by muscle damage, inflammation, fibrosis, early loss of functional abilities, and reduced life expectancy.[2] Extensive fibrosis has been reported to accumulate within the muscles of both mouse models of DMD and Limb girdle muscular dystrophy (LGMD) with aging. Fibrosis in these models is first observed in the diaphragm, followed much later by later involvement in skeletal muscles. Similar to MR observations of the brain, MR contrast and diffusion is often interpreted to represent changes in the underlying cellular structure despite typically acquired at imaging resolutions many times less than that of single cell. In order to directly image muscle cells we adopted a tissue slice set-up developed by Blackband et al[3] to perform MR microscopy (MRM) of brain and spinal cord sections.

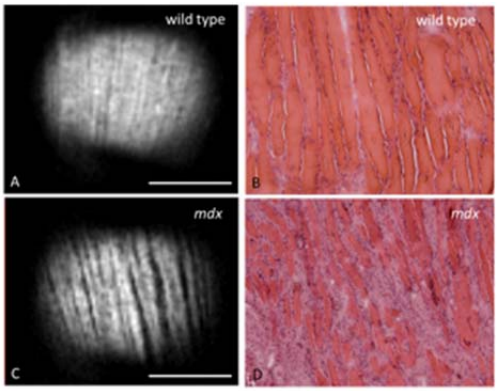

Fig 1: Longitudinal images of Healthy and Dystrophic Muscle

Experimental:

Before MRM, the fixed, mouse diaphragm was washed in PBS overnight to remove formaldehyde and then sliced into sections. In order to obtain the slices used for surface coil MR imaging muscle samples were collected from areas adjoining the central tendon and the coastal margin. The thickness of the sections varied depending upon the application, ranging from 100-250µm. Once muscle sections were collected, they were tailored to fit through gross dissection into the 5mm diameter RF coil sample well with the aid of a dissecting microscope. To make structural validation easier, landmarks such as cells or connective tissues were utilized. To avoid leakage and sample dehydration, the sample was well sealed with PCR film. All MRM was carried out using a 600 MHz Oxford spectrometer equipped with a Bruker Biospin console providing imaging gradient strengths up to 3000 mT/m. For the comparative analysis between wild type and dystrophic (*mdx*) diaphragm muscles, MRM was acquired in both parallel and orthogonal to the striations of the diaphragm.

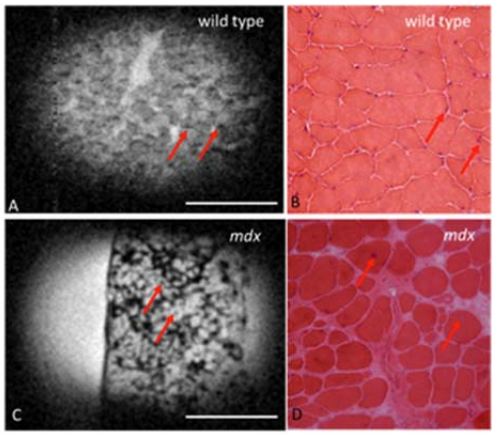

Fig 2: Axial images of Healthy and Dystrophic Muscle

Results and Discussion

Displayed in Fig 1 are longitudinal, two-dimensional MRM of control, unaffected (A) and dystrophic (C; *mdx*) mouse diaphragms with comparative light microscopy of H&E stained control (B) and dystrophic (D) diaphragm sections. MRM images were obtained with a spatial resolution of 15.6x15.6x160 μm^3 within 2 hrs of imaging. Muscle fibers imaged at higher spatial resolutions are shown in Fig 2 in which case the diaphragms have been imaged axially with respect to the muscle fiber long axis at resolutions of = 7.8x7.8x60 μm^3 in 13 hrs and 5 min.

Conclusions:

MRM using microcoils at 14T demonstrate that single muscle cells can be directly visualized with MRI. A mouse muscle cell is typically 30-50µm in diameter and greater than 1 mm in length, well within the spatial resolutions obtained in this study. MRM images also showed the characteristic muscle fiber disarray and infiltration by fibrotic and inflammatory cells associated with dystrophic muscle (*mdx*). MR signal voids around the dystrophic muscle can be attributed to the ultrashort T_2 water species associated with collagen and scar tissue[4].

Acknowledgements:

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References: [1]Hoffman EP et al. Cell. **51**(6): 919,1987. [2] Bushby K et al. Lancet Neurol **9**(1): 77, 2010. [3] Flint JJ et al. Neuroimage. **60**(2):1404,2012. [4] Chang EY et al. JMRI, 2014 (epub ahead of print)