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Muscle fiber types identification by synchrotron fluorescence microspectroscopy

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Keywords : fluorescence microspectroscopy, skeletal muscle fiber type, synchrotron radiation, histology

The skeletal muscle consists of three to four pure types of muscle cells (also called muscle fibers) identified as type I, type IIA, type IIX and/or type IIB in different proportions depending on the muscle function. They differ in their contraction speeds and metabolic pathways. Type I fibers are slow-twitch while type II fibers are fast twitch. The energy required to maintain cell homeostasis and muscle contraction is provided by the hydrolysis of ATP. The fibers IIX and IIB regenerate ATP by anaerobic glycolysis with lactate production. Fibers I and IIA favor cellular respiration (glycolysis + Krebs cycle). The latter are rich in mitochondria, where the cell respiration take place, and in myoglobin which carries oxygen to mitochondria. The intracellular composition of fibers therefore depends on their metabolic and contractile characteristics. The objective of our work was to study the impact of these slight differences in composition on the optical properties of muscle cells. Our hypothesis was, in part, based on the autofluorescence detection of NADH which is more concentrated in the mitochondria and thus in the oxidative metabolism fibers.

Therefore, we studied the impact of cell type on the fluorescent response following excitation in deep UV. Rat soleus muscle consisting of I and IIA fibers and extensor digitorum longus (EDL) muscle consisting of I, IIA, IIX and IIB fibers were used as models. On each muscle, fibers, previously identified on their cell types by immunohistofluorescence, were analyzed by synchrotron fluorescence microspectroscopy on stain-free serial muscle cross sections. Muscle fibers excited at 275 nm showed differences in fluorescence emission intensity among fiber types at 302 (assigned to tyrosine fluorescence), 325, 346 (both assigned to tryptophan fluorescence) and 410 nm (assigned to NADH fluorescence). The 410/325 ratio decreased significantly with contractile and metabolic features in EDL

muscle, ranked I>IIA>IIX>IIB fibers ($p < 0.01$). Compared to type I fibers, the 346/302 ratio of IIA fibers decreased significantly in both EDL and soleus muscles ($p < 0.01$).

In a subsequent experiment, we acquired autofluorescence images for fast fiber types discrimination on label free histological sections. Computer processing of the images allowed us to improve the contrast and identify the metabolic types of fibers with a fairly good reliability.

These studies highlight the usefulness of autofluorescence signals to characterize histological cross section of muscle fibers with no staining chemicals.

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Student: No

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Je suis Cortana. Posez-moi une question.

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