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Characterization of muscle tissue in sepsis induced mouse models

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In order to image deep into native biological tissue to investigate the spatial distribution and organization of specific structures, many researchers rely on the plethora of tissue optical clearing (TOC) methods that are available today. These methods achieve this by reducing the light scattering and light absorption of native tissue by means of refractive index matching, delipidation, and decolorization. However, many of these available methods are only applicable to more homogeneous tissues, such as the brain, as opposed to the highly heterogeneous nature of skeletal muscle. Further compounding the issue, there are no practical substitutes for bungarotoxin (BGT), the most widely used post-synaptic neuromuscular junction marker, which has been found to be incompatible with many of the available TOC protocols. Therefore, the first half of this project focused on creating and validating a TOC method applicable to imaging deep into skeletal muscle, while also maintaining BGT fluorescence. Here, we present MYOCLEAR, a highly robust TOC protocol which enables the analysis of most, if not all, NMJs between adult wildtype and MDX mouse EDL muscles. Though, due to the PFA fixation induced autofluorescence in the lower wavelength channels, this protocol only allows for the evaluation of a single structure and its associated nuclei. To overcome these shortcomings and adapt the MYOCLEAR protocol to faster imaging technuiqes, we have also developed MYOCLEAR+, a PFA free TOC method. This method successfully removes the PFA-induced auto-fluorescence seen in the lower wavelength channels, while also being compatible with light sheet fluorescence microscopy. Though, given the experimental fixation process, protein loss or degradation still need further evaluation. Nevertheless, we also present data which show that denaturing detergents, such as SDS, pH, as well as urea can all effect BGT binding efficacy in a tissue clearing context and should be accounted for when this type of staining is needed.
The second half of this project focused on sepsis, a common life-threating disease with a relatively high mortality rate ranging from 28 – 80%, for systemic inflammatory response (SIRS) and septic shock states, respectively. As many of these patients suffer from vast muscle wasting after a septic state. For the investigation of the effects of sepsis on skeletal muscle, we successfully established a sub-acute/chronic model of sepsis based on the cecal slurry mouse model. Here, we provide evidence of the classical activation of the ubiquitin pathway for protein degradation as well as a significant decrease in both the width and fiber diameter of the diaphragm. Moreover, using activity tracking and western blotting, we provide evidence that not only muscle wasting but critical illness myopathy (CIM) as well as critical illness neuropathy (CIP) induced atrophy could play a role in the case of our model. Lastly, we also provide evidence of the activation and upregulation of fibrosis pathways within the diaphragm. Therefore, we suggest great care to be taken in the future in regards to selecting appropriate housekeeping proteins (HKP) for western blotting as sepsis and fibrosis have been shown to effect many of the routinely used HKP.