Tissue Microstructure Revealed Using Double Pulsed Field Gradient Filtered MRI

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Introduction: Living biological issue, and particularly human tissues and organs are optically turbid. Diffusion MRI methods enable us to learn about tissue microstructure and perform *in situ* and *in vivo* histology. Double Pulsed Field Gradient (d-PFG) NMR multiple scattering techniques[1] in which two diffusion-sensitizing gradients blocks are applied sequentially, can be combined with MRI[2] to glean tissue microstructural information, such as average cell size, shape, and microscopic anisotropy. The diameter of myelinated axons is a crucial neurophysiological parameter that correlates with nerve conduction velocity. In the spine, axons are somatotopically organized into distinct anatomical regions performing specific functions and characterized by different diameters and diameter distributions. The purpose of this study is to evaluate the use of d-PFG MRI[2] to measure and map mean pore or cell diameters within different regions of spinal cord white matter.

Materials and Methods: Formalin-fixed pig spinal cord was rehydrated and put in a 10mm Shigemi tube (Shigemi Inc.) with spinal cord white matter aligned with the z-axis of a 14T vertical-bore Bruker AVANCE III system. The d-PFG NMR parameters were: δ=3.15ms, Δ=60ms, and G was between 0 and 664 mT/m^{-1}; and MRI parameters: TR/TE=3500/6.54 ms, FOV=11mm, matrix size=128x128, and slice thickness=4mm. A recently introduced theoretical framework[3], predicting the MR signal attenuation due to restricted diffusion within a pack of cylinders, was fitted to the data, taking into account a possible free water compartment[4]. A pixel-by-pixel analysis was applied to create a fiber diameter map within the white matter region of the spinal cord. K-means segmentation was performed. For histological studies a portion of the formalin-fixed spinal cord was transferred to 3% glutaraldehyde, post-fixed in osmium tetroxide, and embedded in plastic[5,6]. 1µm-thick tissue sections were stained with toluidine blue. Areas of interest were determined and selected for evaluation on a Zeiss 109 transmission electron microscope.

Results and Discussion: The calculated fiber diameters are in the range expected for such specimen. The clusters produced from the experiment show well-defined regions that generally follow the known anatomy of pig spinal cord white matter. These assignments were verified by histology.

Conclusion: d-PFG filtered MRI is a powerful tool not only for mapping axon diameter, but potentially for other microstructural features of tissues not measurable by other MRI methods.

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