Comparison between histology and MRI markers of white matter damage in contused rat spinal cords treated with transplanted Schwann cells: correlation analysis based on image registration

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TARGET AUDIENCE: Spinal cord injury (SCI) researchers who seek histological validation of MRI biomarkers of white matter injury will find this work relevant.

PURPOSE: MRI techniques have been developed to monitor changes in white matter in SCI; in particular, myelin water fraction (MWF) and transverse diffusivity (D_{trans}) has been associated with myelin sheath integrity[1,2]. We assess the spatial correlation between these MRI quantities and corresponding histology, and determine if the MRI techniques show the same effects as histology under the influence of SKP-SC therapy (transplantation of skin-derived precursors pre-differentiated into Schwann cells), which potentially promote growth and remyelination of axons across the lesion site[3]. Image registration was performed between MRI and histology[4], which allowed correlation between every MR pixel and the area fraction of stain-positive pixels in the corresponding myelin histology area.

METHODS: The spinal cords of 14 Sprague-Dawley rats were contused at the T9/T10 level. At 8 weeks post-injury, cords from 4 animals were perfusion-fixed and the spinal cords excised ("baseline"). Further, 5 animals were injected at the lesion with SKP-SC cells in DMEM media ("cells") at 8 weeks after injury, and 5 animals were injected with media alone ("media") at 8 weeks after injury. The rats of the treated groups were perfusion-fixed and excised at 27 weeks post-injury. Spin-echo DTI and myelin water imaging was acquired with a Bruker 7T preclinical scanner at 5 axial slices spread evenly across the lesion extent (caudal edge, mid-caudal, epicentre, mid-cranial, cranial edge) at 100 µm in-plane resolution and 1mm slice thickness (DTI: TE/TR = 21.3/1500ms, 6 directions, b=750 s/mm2, NA=4; myelin water imaging: single-slice CPMG, TE/TR = 6.738/1500 ms, 32 echoes, NA=6, NNLS analysis). Two stains of transverse sectioned histology slices (20 um thick, 200 um apart within every stain) were used for comparison to MRI: CNS myelin (Eriochrome cyanine or EC) and PNS myelin (P0), imaged at 10x magnification. Affine image registration was performed between MRI and histology as described in [4]. The resultant transformation matrices were used to import the boundaries of the MRI pixel grid into the histology frame to delineate analysis ROIs, in which an area fraction of stain-positive histology pixels was determined by automatic multi-level Otsu thresholding[5]. The EC area fraction was added to the P0 area fraction to form an overall myelin area fraction map ("EC+P0 area fraction") derived from histology, with a one-to-one correspondence to the MRI maps on a pixel-by-pixel basis.

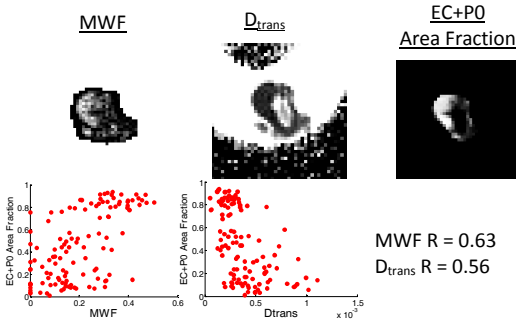

Figure 1. Parameter maps and correlation scatterplots in epicentre slice of a treated cord

RESULTS: Figure 1 depicts the MWF, D_{trans} and EC+P0 area fraction maps from the epicentre slice of a representative cord in the cell group. Note that D_{trans} has the expected negative correlation with myelin content (more intact myelin presents a higher restriction to diffusion across the membrane). The average Pearson coefficient within a slice with respect to EC+P0 area fraction (averaged across the entire study) was 0.45±0.23 for MWF and 0.34±0.19 for D_{trans}. The parameter values were also averaged in radial sectors (dorsal, ventral and lateral) in each slice and were correlated across all animals; the resultant correlations (with Pearson coefficient R and its 95% confidence interval) are shown in scatterplots in Figure 2. MWF correlates better (|R|=0.69) than D_{trans} (|R|=0.49) to the EC+P0 area fraction. Figure 3 shows the overall parameter means and 95% confidence intervals for each experimental group (taken over the pixels over the entire study dataset). Both MWF and EC+P0 area fraction averages were significantly different between each pair of groups (as assessed by Tukey-Kramer test at the threshold p-value of 0.05): a higher myelin content in the transplanted cell group was found versus media alone, while the baseline group shows the highest myelin content. In contrast, the group averages for D_{trans} show no significant difference between groups.

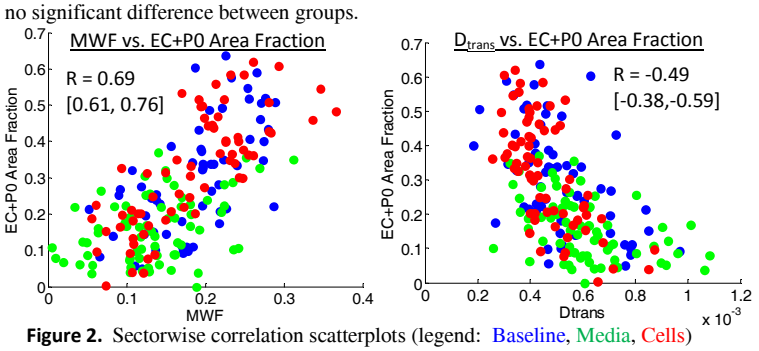

Figure 2. Sectorwise correlation scatterplots (legend: Baseline, Media, Cells)

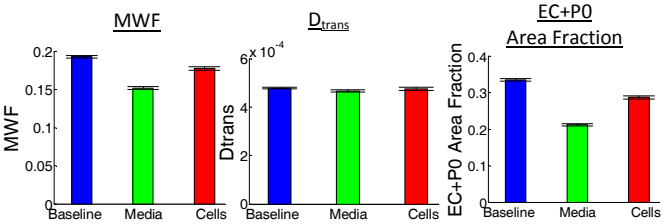

Figure 3. Group averages predicted by MWF, Dtrans and EC+P0 Area fraction (errors bars denote 95% confidence intervals)

DISCUSSION: Myelin water imaging performed well in its ability to correlate with the EC+P0 myelin area fraction, which seems reasonable given that both parameters represent fractional volumes of two closely associated components of the white matter (water trapped between myelin and the myelin itself, respectively). Both modalities show the desired effect of treatment (higher myelin content), but show the unexpected result that the baseline group had the highest myelin content (this may be due to both continued primary demyelination past 8 weeks, and the presence of myelin debris at 8 weeks which would be largely cleared by 27 weeks[1]). The correlation may have worsened due to segmentation errors in the histology quantification (relatively low magnification which may have introduced blurring), and an erroneous lack of MWF contrast between the white matter rim and the central gray matter/lesion which sometimes appeared, especially in the baseline group. The cause of this discrepancy is unknown, but any biological phenomenon which could shorten the T2 relaxation time of water could result in a mistaken increase in MWF. In contrast, D_{trans} did not correlate as strongly with EC+P0 area fraction nor did it show the same trends as clearly. This is perhaps not surprising since the myelin area fraction is qualitatively different than what D_{trans} measures, which is the ability of the myelin sheath to resist water diffusion across the membrane. Even this meaning is only strictly valid under a set of conditions (e.g. uniform pixel composition, parallel fibres[6]) which may not hold true in the injury lesion.

CONCLUSION: MWF was shown to correspond highly to a histological measure of myelin content in a common SCI animal model, both in its spatial correspondence and ability to predict the same effects of a cell transplant treatment. D_{trans} correlated less with respect to EC+P0 area fraction, and showed no significant effects of treatment. Image registration was a key tool in enabling this correlation analysis by preserving much of the pixelwise spatial information that would be lost by a more conventional ROI-based approach. **Acknowledgments**: This work was supported by Canadian Institutes for Health Research and National Sciences and Engineering Research Council of Canada. **References:** 1. Kozlowski et al, J Neurotrauma 2008:653-76. 2. Tu et al, NMR in Biomed 2013:1484-95. 3. Biernaske et al, J Neurosci 2007: 9545-59. 4. Yung et al, ISMRM 2014: 1729. 5. Liao et al, J Inf Sci Eng 2001:713-27. 6. Nilsson et al, Magn Reson Mater Phy 2013:345-70.