Ex vivo myelin water and DTI measurements of SKP-SC transplanted cell therapy in contused rat spinal cord: correlation with histology

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Introduction Schwann cells differentiated from skin-derived precursors (SKP-SCs) have been shown to promote histological and functional recovery in a contusion model of rat spinal cord injury [1]. Cellular bands of SKP-SCs bridge the lesion site which promote axon growth ensheathed by peripheral (Schwann cell) myelin of endogenous as well as transplant origins. In this work, we assess the myelin content and axonal integrity in an ex vivo specimen of contused thoracic cord treated with SKP-SCs by measuring myelin water fraction (MWF) [2] and longitudinal diffusivity (D_{long}), and compare them to corresponding histological measures.

Methods For each rat (male Sprague-Dawley, N=9), one million SKP-SC cells were transplanted into the lesion site 8 weeks after contusion injury at T9/T10 (IH impactor, 200 kdynes), and were sacrificed and perfusion-fixed (4% PF) at week 27 post injury. A 4 turn solenoid (i.d. = 1.2 cm) was used to acquire spin echo DTI data (TE/TR = 21.3/1500 ms, 6 directions, b=750 s/mm^2, NA=4, FOV=1.28x1.28 cm, matrix=128x128) and CPMG data (TE/TR = 6.738/1500 ms, 32 echoes, NA=6, FOV=2.56x2.56, matrix=256x256). T_2 distributions were generated from the CPMG data on a pixelwise basis using non-negative least squares analysis [3], and myelin water fraction was calculated as the fractional integral of the signal with T_2 values between 7.75-20 ms. Immunohistochemistry was performed using fluorescence microscopy after sectioning (20 um thickness) to examine axons (NF/Tub: neurofilament-200/β3-tubulin stain) and myelin content (MBP/P0: all myelin and peripheral myelin, respectively). The histology images were thresholded manually to remove background pixels, and the fraction of pixels above threshold in histology (NF/Tub or MBP/P0) was compared to the average value of the corresponding MRI measure (MWF or D_{long}) within ROIs encompassing the entire tissue space excluding the lesion cavity.

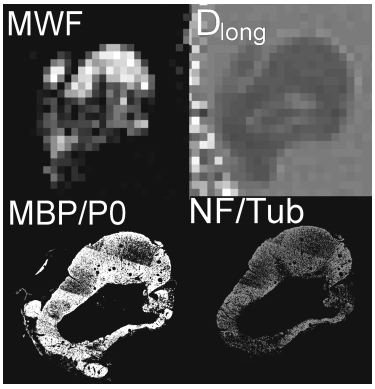

Figure 1. *Maps of MWF, D_{long}, MBP/P0-positive pixels, NF/Tub-positive pixels in the centre of a example spinal cord specimen*

Results and Discussion Figure 1 shows MWF, D_{long}, NF/Tub-positive pixels and MBP/Po-positive pixels at the centre of a typical lesion. Datasets from two specimens were not used due to inconsistent gain/offset correction in the histology images and artifactual MWF maps. Figure 2 shows excellent correlation between cavity sizes as measured by MRI and histology (R=0.99). In addition, Figure 3 illustrates that the average MWF per cord exhibited a strong correlation with the percentage of MBP/P0 pixels above threshold (R=0.87), suggesting that MWF is a sensitive measure of myelin content. Figure 4 shows that the correlation between D_{long} and the percentage of NF/Tub-positive pixels was markedly lower(R= 0.32), which may be due to D_{long} being unable to distinguish intact axons with the less restricted space of damaged axons (ie. Dlong will remain high in both cases). It is interesting to note that the MBP/P0 stain is able to differentiate between the original CNS myelin (MBP-positive only) and the peripheral myelin that is generated in the lesion due to the transplanted cell therapy (P0-positive). Further data analysis will focus on image registration between MRI and histology to allow detection of differences in the MRI measures between these two myelin populations.

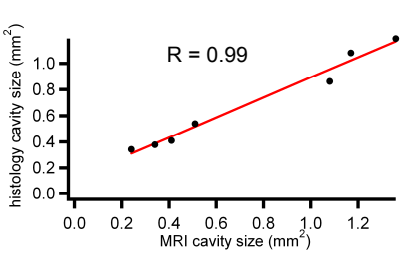

Figure 2. *Cavity area defined by MRI vs.cavity area defined by histology images*

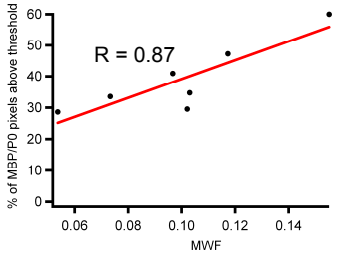

Figure 3. *Average myelin water fraction vs. percentage of MBP/P0-positive pixels*

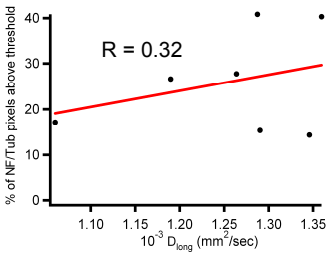

Figure 4. *Average longitudinal diffusivity vs. percentage of NF/Tub-positive pixels*

Conclusion This preliminary work shows that the structural effect of SKP-SC therapy in rat cords is measurable by examining lesion size, myelin water fraction and longitudinal diffusivity ex vivo, which may constitute a first step towards monitoring the effect of this therapy *in vivo* using MRI. **Acknowledgments** This study has been supported by the Canadian Institutes of Health Research.

References [1] Biernaske et al., J Neuroscience, 27(36):9545-59, 2007. [2] Kozlowski P et al., J Neurotrauma, 25(6) :653-76, 2008. [3] Whittall et al., Magn Reson Med, 37(1):34-43, 1997.