Magnetic Resonance Molecular Imaging of Neural Sprouts in Spine Explant Cultures

I. E. Chesnick[1], C. B. Fowler[1], Y. Kim[2], H. E. D'Arceuil[3], J. T. Mason[1], and K. Potter[1]
[1]Department of Biophysics, Armed Forces Institute of Pathology Annex, Rockville, MD, United States, [2]Defense and Veterans Brain Injury Center, Armed Forces Institute of Pathology Annex, Rockville, MD, United States, [3]MRVision Co., Redwood City, CA, United States

Introduction: There are several spinal cord injury models available for studying axonal repair in vivo [1] however, these studies are restricted to functional or behavioural changes [2] or tissue level pathologic changes detectable by MRI [3] and by histology [4]. In vitro models that use dissociated neurons provide useful information about extrinsic factors and substrates that promote neural cell differentiation and neural sprouting but are of limited value in studies of axonal regeneration. In contrast, organotypic slice cultures of spinal cord allow for the study of axonal growth and regeneration, synapse formation, and myelination [5]. Consequently, to better understand the dynamics of spinal cord repair in vivo, we used spinal cord explant cultures to test the specificity of actin-targeted Gd-liposomes to actin-rich neural sprouts. This model system provides a novel test platform for the development of MR molecular imaging probes for the study of unique cell populations, synapse formation, and axonal regeneration in models of spinal cord repair.

Materials and Methods: Spinal cords, including the brachial, thoracal, and lumbar regions were dissected from day 7 chick embryos [6]. Cords were removed from the vertebral column and dissected free of ganglia and meninges and washed with ice-cold culture medium. The spinal cord was split at the midline creating left and right halves. Spinal explants were attached to glass slides with fibrin and maintained in culture for 1 week. Evidence of neural sprouting was confirmed by light microscopy and explants were subjected to MRM post-fixation. MRM images, with a nominal in-plane resolution of 94 μm, were acquired on a Bruker DMX spectrometer operating at 9.4T equipped with a micro-imaging gradient set and a custom built triple-tuned (^{1}H, ^{31}P, and ^{19}F) probe (Bruker Biospin) for imaging thin tissue sections. High-resolution T1-weighted anatomic images were acquired pre- and post-treatment with phalloidin labelled Gd-liposomes containing rhodamine. The localization of Rd-Gd-liposomes was confirmed by confocal microscopy.

Results and Discussion: There was significant signal enhancement on T1w images of cells labeled with actin-targeted Gd-liposomes (Fig 1A) compared to cells treated with fluorescently-labelled actin (Fig 1B). Fluorescence images of the respective wells confirmed the labeling of the cells with Rd-Gd-liposomes (Fig 1C) and with Alexa Fluor®488 (Fig 1D). Using the same labeling technique on spine explants, we found that there was significant signal enhancement on T1w images along the mid-line of the explant, where the neural tube is located, between spinal segments, and at the explant edges after treatment (Fig 1E) with actin-targeted Rd-Gd-liposomes compared to pre-treatment images (Fig 1F). Using light microscopy, we found that many neural sprouts extended out from spinal segments (Fig 1G) and confocal imaging confirmed the localization of Rd-Gd-liposomes to neural sprouts on the edge of the explant. In conclusion, we have succeeded in amplifying the MR signal from actin-rich neural spouts using actin-targeted Gd-liposomes. Future work will include the use of additional targeting antibodies to confirm the utility of MR molecular imaging for axonal regeneration studies.

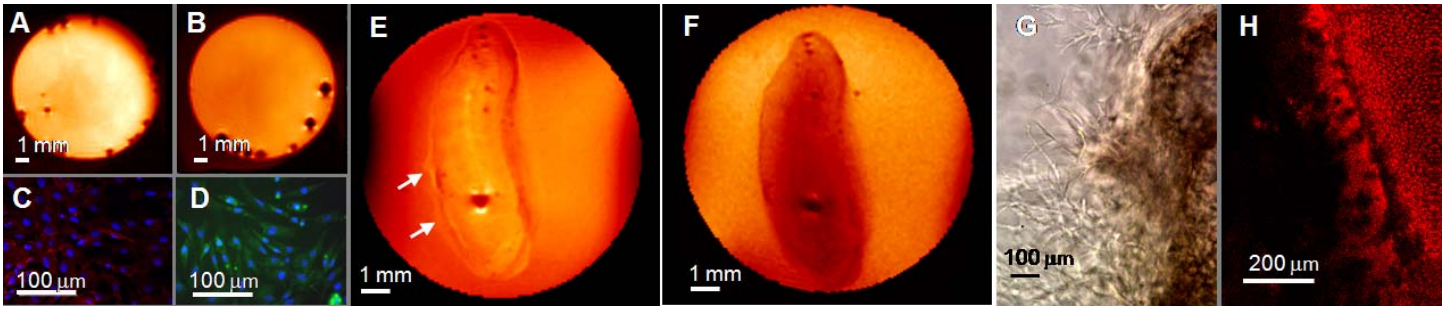

Figure 1. T1w images of osteoblasts grown on circular coverslips treated with phalloidin labeled with (A) Rd-Gd-liposomes and (B) Alexa Fluor® 488. Fluorescence images confirm the staining of actin fibers with (C) Rd-Gd-liposomes (red) and (D) Alexa Fluor® 488 (green). Nuclei were stained blue by DAPI . T1w images of spine explants post- (E) and pre-treatment (F) with Rd-Gd-liposomes show signal enhancement around neural sprouts (white arrows) seen in bright field (G) and confocal (H) images. Dark spots seen on MRM images are from air bubbles.

Acknowledgements: The authors would like to thank Parul Gangwal for her assistance in preparing the liposomes.

R**eferences:1.** Rosenzweig, E.S. and J.W. McDonald, Curr Opin Neurol, 2004. 17(2): p. 121-31. **2.** Basso, D.M., et al., *s.* J Neurotrauma, 2006. 23(5): p. 635-59. **3.** Kim, J.H., et al., Magn Reson Med, 2007. 58(2): p. 253-60. **4.** Ma, M., et al., 2001. 169(2): p. 239-54. **5.** Thomson, C.E., et al.,. J Neurosci Res, 2006. 84(8): p. 1703-15. **6.** Rosner, H. and G. Vacun,. J Neurosci Methods, 1997. 78(1-2): p. 93-103.