## Development and Stability Testing of an MRI Compatible Isolated Tissue System

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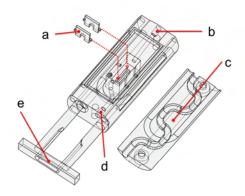


Figure 1: MRI compatible incubation chamber (exploded), produced in nylon using 3D selective laser sintering. Circular diameter of the main chamber = 26mm. a) inserts for grease-gap electrophysiology which sit over the central bench to support the optic nerve, b) medium inflow, c) preheating system in lower section of chamber, d) medium outflow and e) tubing support and isocentre positioning bar.

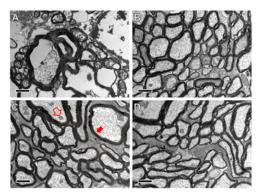


Figure2: Transmission electron microscope images at 20k of four treated optic nerves, a) oxygen and glucose chamber. Open arrow shows a healthy mitochondia, the solid arrow shows compact myelin layers surrounding an axon. Scale bars =  $1\mu m$ .

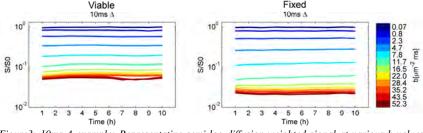


Figure 3: 10ms  $\Delta$  example: Representative semi-log diffusion-weighted signal at various b-values from diffusion MR experiments on a viable (left) and a fixed (right) optic nerve. Both nerves were scanned with range of diffusion weightings once per hour  $\Delta = 10$ ms for 10 h.

Synopsis: We have developed and validated an MRI compatible chamber to maintain isolated tissue in a structurally and functionally viable and stable condition for acquisition of detailed MRI data sets. We demonstrate that rat optic nerve tissue is structurally and functionally stable within our chamber for 10 h, reflecting the in vivo conditions of the nerve. We use electrophysiology, MR diffusion measurements and electron microscopy to measure the nerve's condition and performance over time. The design offers new opportunities to study viable tissue for example to develop and validate models of diffusion in tissue.

Introduction: In vivo MRI assessment of neuronal tissue whether clinical or experimental models is complicated by movement, surrounding cell types, vascular and susceptibility effects and restricted scan duration. These confounders limit the accuracy of MRI data. Encouraging attempts to mitigate the issues with in vivo MRI have been made in pre-clinical work: Artificial phantoms can provide a stable and simple system e.g. [1] but cannot replicate the structural complexity of in vivo tissue nor its functional behaviour in any detail. Chemically fixed tissue provides a close model of the level of structural complexity seen in vivo but fixation alters structure and affects the chemical environment and behaviour of the water altering MRI measurements [2&3]. However, viable isolated tissue systems potentially fill the gap, providing a close model of in vivo tissue with the stability of isolated tissue. Viable isolated tissue maintained in an organ bath allows physiological changes to be investigated and more closely reflects in vivo tissue than systems based upon fixed tissue. Using isolated tissue allows more lengthy scans than are feasible in vivo enabling in depth exploration of MRI parameter space or isolated functional studies.

We have developed an MRI compatible viable isolated tissue system to investigate nervous tissue over many hours. This work demonstrates the system's ability to maintain viable tissue stability over 10 h at body temperature. For testing we use rat optic nerve, which is a well characterised and consistent tissue [4]. The chamber described here provides a novel, controllable and versatile imaging system which can be used with horizontal bore MRI systems.

**Methods:** The MR compatible incubation chamber shown in Figure 1 was produced using high resolution selective laser sintering on a Formiga P100 SLS System (EOS). Tissue is maintained at 36.5°C within the chamber and constantly perfused with oxygenated artificial cerebral spinal fluid (aCSF) (1ml per min). In order to assess the functional stability of the optic nerve over time, grease gap electrophysiology was used [5]. Structural assessment of the optic nerve cells over time has been conducted using transmission electron microscopy (TEM). To assess MRI stability, diffusion MR measurements of the nerve (9.4T Agilent VNMRS system) were taken: 1D imaging (128 data points) with orthogonal slice selective excitation and refocusing pulses were used. 48 diffusion weighted images were acquired per hour in 4 sets of 12, a set for each of the 4 diffusion-sensitizing gradient separations ( $\Delta$ ) deprived for 2 h. b) immediately fixed nerve. c) aCSF for 10ms, 20ms, 35ms and 50ms. For all measurements, diffusion encoding gradients were 5 h within the chamber, d) aCSF for 10 h within the aligned orthogonal to the nerve and their amplitude was increased from 0 to a maximum of 950 mT/m.  $\delta = 3$ ms, TE= 24, 34, 49 and 64ms, respectively (minimised allowing for  $\Delta$ ) TR = 3700ms. This pulsed gradient spin echo (PGSE) protocol is similar to that used for detecting

diffusion differences in viable and fixed rat brain slices as in [2].

Results: Action potentials generated from the nerve showed no significant variation over 10 h within the chamber (data not shown here). There was no loss of structural integrity of the axons within the nerve over time (0, 5 and 10 h). Figure 2 shows TEM images demonstrating this stability. Viable tissue showed consistently higher diffusion weighted signal than fixed tissue. Crucially comparable variability and stability is apparent in both tissues over time (Figure 3). This pattern is consistent across the other three  $\Delta$  values measured (not shown).

Discussion: In this work we demonstrate the structural, functional and MR stability of rat optic nerve within this MRI compatible tissue maintenance chamber over time. We hope that this well validated system may become a standard model for examination of viable isolated tissue: finding uses for validation of quantitative MRI (for example, contrast agent uptake, axon diameter measurements and tractography) and will be of particular use in testing of diffusion MRI white matter models (reviewed in [6]). In contrast to other recent viable excised tissue models, this system: 1) Operates and maintains the tissue temperature at  $36.5 \pm 0.5^{\circ}$ C, 2) Is validated for 10 h with electron microscopy, electrophysiology and diffusion MRI, 3) Is a reliable, reproducible high field MRI compatible piece of equipment which could be used in various MRI scanners.

Selected references: [1] Perrin, M. et al. PHILOS T R SOC B, 360, 881-891 (2005). [2] Shepherd, T.M. et al. MAGN RESON MED, 62, 26-34 (2009). [3] Alexander, D.C. et al. NEUROIMAGE, 52, 1374-1389 (2010). [4] De Juan, J. et al. ACTA ANAT, 102, 294-299 (1978). [5] Garthwaite, G. et al. - EUR J NEUROSCI, 11, 4367-4372 (1999). [6] Panagiotaki, E. et al. NEUROIMAGE, In press (2011), doi:10.1016/j.neuroimage.2011.09.081. Acknowledgements: Peter Johnson, Dr Giti Garthwaite, Professor John Garthwaite. Funding: MRC Capacity Building Studentship.