Perfluoropolyethers in Magnetic Resonance Microscopy: Effect on Quantitative Magnetic Resonance Imaging Measures and Histological Properties of Formalin-Fixed Brain Tissue

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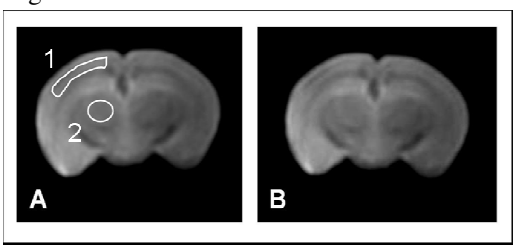
Introduction: Magnetic resonance microscopy (MRM) allows non-invasive characterisation of tissue structure at resolutions approaching those achievable by histological methods. With the availability of new murine genetic models of human diseases, MRM is being used increasingly for morphological and metabolic phenotyping. *Ex vivo* MRM requires tissues to be immersed in a suitable medium to prevent sample dehydration and the generation of susceptibility artefacts arising from tissue-air interfaces. Perfluoropolyethers (PFPE) such as Fomblin® (Ausimont, Thorofare, NJ) have been used in a number of studies as a wetting and embedding agent for *ex vivo* MRI of biological tissues[1-6]. The use of PFPE is near ideal having few ^{1}H nuclei, is non-toxic and non-inflammable. The purpose of this study was to determine whether prolonged immersion in PFPE alters MRI properties and/or the histological properties of formalin-fixed tissue.

Methods: To assess the effect of prolonged PFPE immersion on histology, 8 *CD1* mouse brains were formalin-fixed for 2 days, after which 4 were immersed into Fomblin® for 48 hours and 4 remained in 10% formol-saline as controls. After 2 days, routine histological analysis including immunohistochemistry was performed by a consultant neuropathologist. To assess the effect of PFPE on MR parameters, a further 8 *CD1* mouse brains were formalin-fixed for 10 days, after which half were immersed into Fomblin® and half remained in formol-saline (controls). After 2 days all specimens were transferred to Fomblin® for MRI at 9.4T. T1-, T2-, diffusion-weighted, and magnetisation transfer ratio (MTR) imaging was performed and maps of quantitative MR measures generated. Regions of interest (ROIs) were defined in the right thalamus and right cortex (see Fig 1A). The Mann-Whitney U test was used to assess differences in ROI MR values between the two groups with $p<0.05$ considered statistically significant. After MRI, the mouse brains underwent histological analysis as above.

Results: Visual inspection of high resolution T2-weighted MRI images of control and Fomblin®-immersed mouse brains revealed no discernible differences between the groups brains (see Fig 1A and B). There were no significant differences in any quantitative MR measure between the control and Fomblin-immersed groups (see table for right thalamus ROI values). Histological investigation revealed no visible difference in anatomical or cellular detail or immunohistochemical staining between the 2 groups (see Fig 2 A-E).

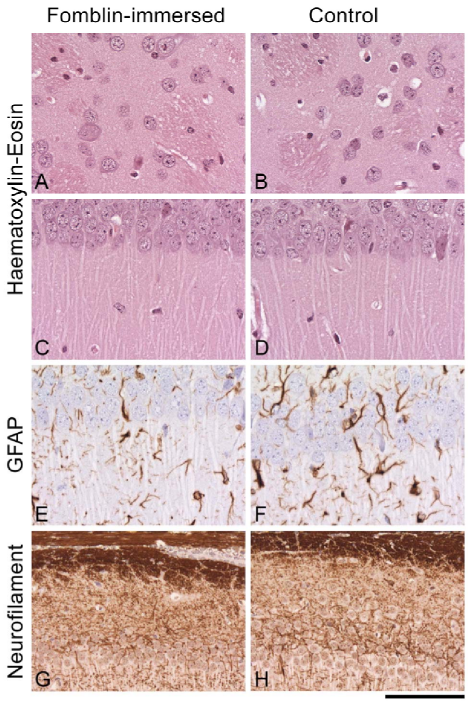
Conclusions: MRM allows living organisms and fixed tissue to be imaged clearly and non-destructively. Ex-vivo MRM of specimens is frequently used as an investigative tool following *in vivo* imaging of animal models of disease for histopathological correlation, and for this tool to be accurate, it is important that sample preparation and handling does not alter either the MRI or the histological properties of the tissue. In conclusion, immersion of formalin-fixed tissue into PFPE for extended periods of time had no discernible effects on histological appearances and, within our experimental accuracy did not alter MRI relaxation, diffusion or MTR properties, confirming its suitability for use as an embedding medium for MRM of fixed tissue.

Figure 1



	Right thalamus ROI		
	Control	Fomblin	*p*
T1(ms)	1499.7(85.1)	1505.3(93.0)	0.87
T2(ms)	34.8(4.4)	33.6(3.1)	1.00
MTR	62.6(5.4)	60.6(7.2)	0.87
MD	339.3(59.8)	308.7(93.4)	0.69
FA	0.35(0.06)	0.41(0.11)	0.34

Figure 2



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