Dichromate as an MR Microscopy Stain

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Introduction

Unlike optical microscopy, MR Microscopy (MRM) has few stains available to enhance contrast between tissue types. A significant proportion of MRM studies use clinically available gadolinium-based contrast agents to shorten T1 relaxation times, enabling increased SNR. This non-specific staining may not, however, generate increased tissue contrast within the specimen. Luxol fast blue [2], a paramagnetic copper phthalocyanine that binds to myelinated axons throughout the central nervous system, is one of limited compounds that does show the required specificity for highlighting the tissue of interest. Recently, potassium dichromate has been shown to enhance tissue contrast in fixed brain specimens. Whilst these studies were performed at high field (7T [2] and 11.7T [3]), it has also been shown to work at lower field (4.7T, [4]). Here, at comparatively low field (4.7T) and using laryngeal specimens, the general applicability of potassium dichromate as a tissue specific stain is demonstrated. Tissue specific generation of paramagnetic chromium species provides T1 based enhanced signal and tissue contrast. Furthermore, following MR imaging, it is shown that specimens may be processed for histology using normal procedures and that the MR staining procedure does not affect the optical–based staining.

Methods

Larynges from three beagles that had been humanely sacrificed for extraneous studies were sectioned to provide 6 individual specimens. The specimens were placed into 10% neutral buffered formalin (NBF) for 24 hours, washed with RO water, immersed in Fluorinert and MR images acquired before staining. Tissues were stained with an aqueous solution (5% w/v) of potassium dichromate at 4°C for 48 hours and subsequently washed with RO water, immersed in Fluorinert and MR images acquired. MR images were obtained at 4.7T using a Varian Inova imaging and spectroscopy system utilizing a Varian 3 cm inner diameter quadrature coil. A 3D T1-weighted gradient echo sequence (TR=20.6 ms; TE=6.5 ms; FL = 65°; FOV= 60 x 15 x 15 mm; MA = 256 x 128 x 128; NT =4) was used to acquire lower resolution images (~117 μm isotropic resolution, figure 2A&B) whilst the same parameters (except MA=512x256x256, NT=16) were used to acquire higher resolution data (~59 μm isotropic resolution, figure 3A). Following completion of the MR studies, specimens were returned to 10% NBF and stored for 7 days before standard histological processing and H&E staining.

Results

In all specimens investigated, negligible tissue distortion resulted from immersion in dichromate solution (figure 1). MR imaging data showed dramatically enhanced image contrast between differing tissue types (figure 2). Increased signal was also observed for the majority of tissue types except, for example, lipid which became hypointense following staining (figure 2B). Individual muscle fibers could be readily observed post staining (figures 2B&3A). Standard H&E staining appeared normal and unaffected by the chromium-based staining procedure (figure 3B).

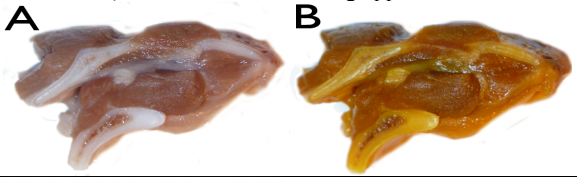

Figure 1: Photographs of a typical specimen (A) before and (B) 48 hours after immersion in an aqueous 5% w/v potassium dichromate solution showing dichromate-induced yellow colouration and negligible sample distortion.

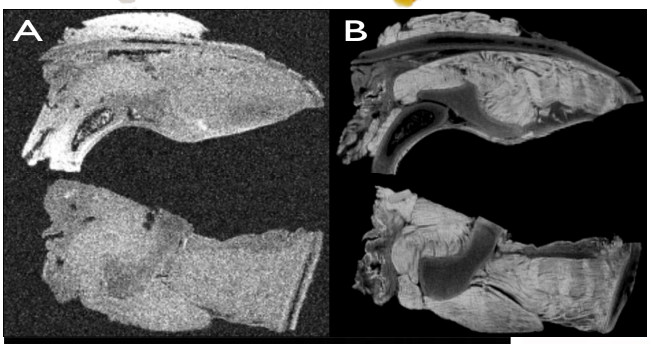

Figure 2: Representative 117 μm slices from the T1W GE3D data acquired from a dog laryngeal specimen (A) before and (B) after staining with 5% (w/v) potassium dichromate. In this and all specimens investigated, significantly enhanced signal and contrast was observed. Lipid appeared hyperintense before staining but became hypointense post staining. Individual muscle fibers could be seen post staining presumably due to specific tissue staining of fibroconnective tissue. Cartilage also showed enhanced intra-tissue contrast exhibiting layers that may correspond to perichondrium tissue. The same specimen is shown at higher resolution in figure 3A.

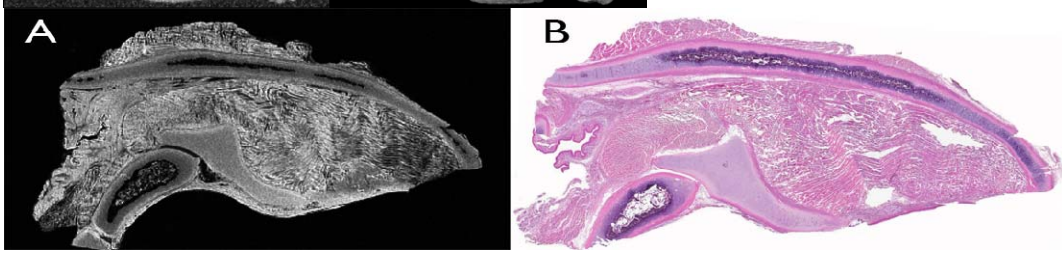

Figure 3: An MR image, from the same specimen shown in figure 2, with a slice thickness of 59 μm is shown in (A) together with (B) an equivalent 5 μm H&E stained histology slice from the same specimen showing that normal histology is unaffected by the MR staining procedure.

Discussion and Conclusion

The use of potassium dichromate as a tissue specific MR histology stain is demonstrated in a range of differing tissue types. The MR imaging data shown here clearly reveals enhanced SNR for a number of different tissues, lipid being a notable exception. Enhanced SNR facilitates high-resolution studies enabling relatively low field systems (4.7T) to acquire MR microscopic data. Furthermore, this study also demonstrates that despite tissue oxidation yielding paramagnetic species ultimately responsible for the enhanced signal and contrast, standard histological procedures are unaffected and may be performed following staining and MR investigation.

References

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[4] Proc IEEE Int Symp Biomed Imaging,742-745 (2011)