

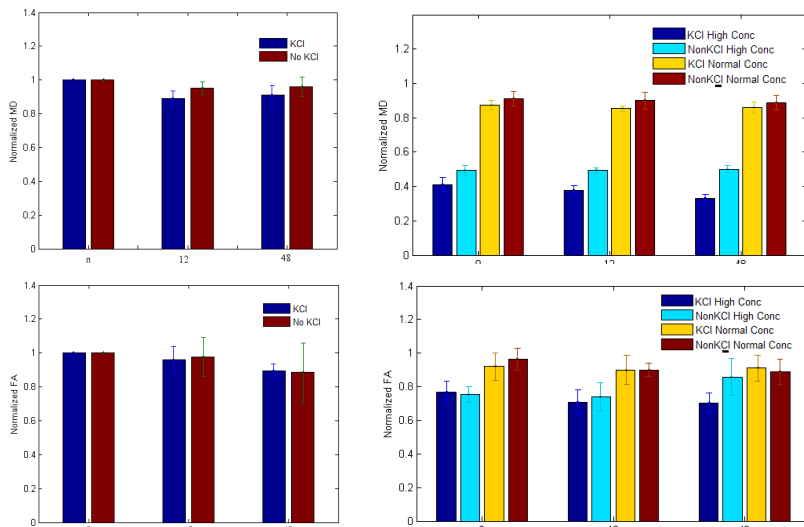
## Effects of Formalin Fixation on Diffusion Tensor Imaging of Myocardial Tissues

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**Introduction:** Diffusion Tensor imaging (DTI) [1] has emerged as a promising means to noninvasively or nondestructively characterize fiber and laminar structures of the myocardium [2,3]. Because of the prolonged scan time required, high-resolution or 3D DTI experiments are often performed on specimens that are chemically fixed. Although the impacts in brain tissues have been studied [4,5,6], little is known regarding the effects of fixation on DTI of cardiac specimens. The knowledge is important for both proper experimental design and accurate interpretation of the results. The goals of the current study is thus to systematically assess the effects of fixation, including factors such as specimen preparation, time of fixation, fixation duration, and fixative type, on DTI derived parameters measured in myocardial specimens.

**Methods:** Freshly-excised intact porcine hearts (n=7) were obtained from an unrelated study [7]. Some of the hearts (n=4) were retrogradely-perfused with KCl to put the hearts into diastole. The equatorial region of each heart left ventricle was dissected into 16 wedges and scanned immediately (t=0). In addition to being KCl or non-KCl treated, the wedges (n=112 total) were randomly assigned to be exposed to either normal (10%) or high (37%) concentration formalin for fixation. Specimens in each treatment type (e.g., non-KCl, normal formalin) were subdivided into three groups corresponding to different starting times of fixation (immediately,  $12 \pm 4$ , or  $48 \pm 5$  hrs after initial scan). Specimens in the t=48 hr group were also scanned at 12 hours and 48 hours (right before fixation) and stored in saline under 4°C refrigeration between scans. All fixed specimens were scanned at t=7 days after initial scan. Two-dimensional DTI scans were performed in the mid-transverse plane of the specimens on a Bruker 7T Biospec MRI instrument using a multi-slice spin-echo diffusion sequence encoded in 12 gradient directions, slice thickness=1 mm, in-plane resolution=300  $\mu$ m, TR=1500 ms, TE=18 ms, b=1500 s/mm<sup>2</sup>. Diffusion tensors were estimated as described previously [2] and used to compute the mean diffusivity (MD) and fractional anisotropy (FA). All DTI parameters were normalized to their respective values obtained at t=0. Two-way ANOVA, as functions of time and KCl treatment, was performed on each of the DTI parameters obtained on unfixed specimens. Likewise, three-way ANOVA, with fixative concentration as the additional variable, was performed on the post-fixation data.



**Figure 1.** Normalized DTI mean diffusivity (top) and FA (bottom) obtained from unfixed (left) and fixed (right) specimens, plotted as functions of treatment type as specified in the legend. The horizontal axis for the unfixed case is the pre-fixation storage time, whereas that for the fixed specimen case is time to fixation, in hours.

of fixed myocardial specimens.

### References:

[1] Basser PJ et al, Biophys J, 66: 259, 1994. [2] Hsu EW et al, Am J Physiol, 274: H1627, 1998. [3] Helm PA et al, Magn Reson Med, 54: 850, 2005. [4] Widjaja E et al, Magn Reson Img, 27: 865, 2009. [5] Shephard et al, Magn Reson Med, 62: 26, 2009. [6] Thelwall et al, Magn Reson Med, 56: 282, 2006. [7] Vergara GR et al, Heart Rhythm, Vol 8, Issue 2:295.

**Results and Discussion:** Figure 1 shows bar graphs summarizing the normalized DTI MD and FA obtained as functions of storage time or time of fixation and treatment type. Results of the ANOVA tests indicate that for unfixed specimens, no significant differences were observed for MD or FA as functions of storage time or KCl treatment, except for the single case of FA in KCl-treated specimens at 48 hrs. For the fixed specimens, fixation with high concentration formalin dramatically and significantly decreases the MD, which is amplified by KCl treatment. No time-dependence were found for the MD, although all values were significantly lower than the initial t=0 value. In contrast, only significant dependence for FA was found for the fixative concentration. The differing responses of the MD and FA likely reflect the nature of fixation cross-linking and redistribution of tissue water.

**Conclusion:** Formalin fixation causes significant decrease in the measured DTI diffusivity and FA that depend on the concentration of the fixative used. However, after initial change, the DTI parameters remained stable over time, at least up to 7 days post fixation. No significant effect was found for KCl treatment. These findings provide useful guidelines for specimen preparation and experimental design for DTI