Multiexponential T2 Analysis of Astrocytoma Cells in Agar

P. R. Jackson[1], R. G. Henry[2], and T. R. McKnight[2]

[1]Joint Graduate Group in Bioengineering, University of California, Berkeley and San Francisco, San Francisco, CA, United States, [2]Department of Radiology, University of California, San Francisco, San Francisco, CA, United States

Introduction

A well-known characteristic of malignant glioma is an increase in cell density with increasing malignancy of the tumor. In order to tease out which T_2 components correlate with cell density, we created a simple model for tissue using astrocytoma cells suspended in agar at different densities to better understand the effects of cell density.

Methods

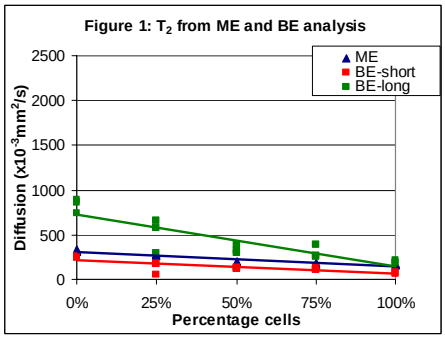
SAMPLE PREP: Cultured genetically engineered astrocytoma cells[1] were trypsinized, washed in D_2O based Phosphate Buffered Saline (D-PBS), and centrifuged at 400×g at 5°C. Two percent (w/v) low melting point agarose (Sea Prep, Lonza, Basel, Switzerland) was made with D-PBS and TSP/D_2O. Cells and agarose were mixed to create samples that were 0%, 25%, 50%, 75% and 100% cells (N=3 each). The cell and agarose mixtures were loaded into a dual-open-ended 5mm NMR tube with susceptibility matched plugs (New Era, Vineland, NJ). Each sample was placed on ice and allowed to gel for at least 2 hours prior to performing NMR measurements.

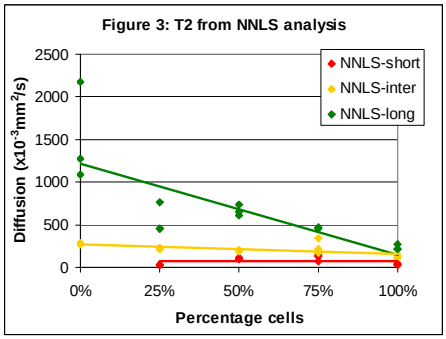
NMR MEASUREMENTS: All NMR experiments were performed at 10°C on a 500MHz Varian spectrometer. CPMG experiments were executed using 64 TEs linearly spaced between 10ms and 1270ms, TR=10s, NEX=2.

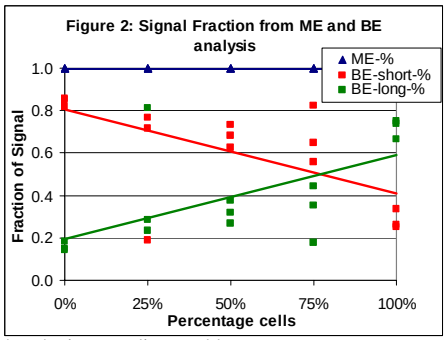
DATA ANALYSIS: The water peak heights were measured at 4.9ppm using ACD (Toronto, Canada) and were used to construct T_2 decay curves. The resulting exponentials were analyzed using monoexponential (ME), biexponential (BE), Non-Negative Least Squares (NNLS) [2,3] algorithms in MATLAB (MathWorks, Natick, MA). The general fit equation was

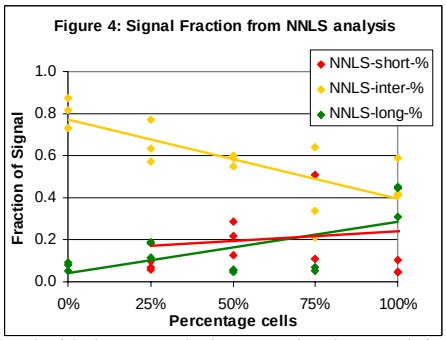
$$S/S_0 = \sum_{a=1}^{n} f_a e^{-\frac{TE}{T_{2\,a}}}$$

where S/S_0 is the normalized signal intensity, T_2 is the transverse relaxation, f_a is the fraction of signal associated with a particular T_2 assuming no exchange, TE is the echo time, and n is the number of components. For monoexponential fits n=1, and for biexponential fits n=2. The variable n was not assigned *a priori* for NNLS, but was one of the fit parameters. Only components that contributed at least 5% to the signal ($f_a \geq 0.05$) and had a value of at least 25ms were assumed to be true components rather than errors arising from noise in the model. Spearman Rank correlation with a significance level of $p \leq 0.01$ was used to detect correlations with cellularity while controlling for multiple comparisons.









Results

In Figure 1, the ME T_2 fit (blue)decreased linearly ($p<.001$) with increasing cell content. For BE analysis, both the long (green) and short (red) T_2 components decreased increasing density ($p<.001$ and $p=.001$, respectively). Interestingly, the correlation coefficients for the long and short signal fractions were identical and opposite one another ($r_{short}= -0.66$ and $r_{long}= 0.66$, $p=0.008$). The short signal fraction dominated for all but the 100% sample.

The NNLS analysis (Figure 3) fit two T_2 components for 0% sample and three components for all other samples. Both the long ($p=0.001$),and the intermediate (yellow, $p=0.001$) T_2 component decreased with increasing cell density, while the short ($p=0.84$) component remained constant. In Figure 4, the intermediate T_2 signal fraction composed a majority of the signal for all sample types and decreased with cell density($p=0.002$). The long ($p=0.21$) and the short ($p=0.84$) signal fractions did not change significantly.

Discussion

For the ME analysis (Figure 1), the T_2 shortened with higher cell densities. The same observation was seen in the short and long components of the BE analysis. In Figure 2, the long and short fraction exactly opposed each other with increasing cell density.

For NNLS, the short T_2 component (Figure 3) was most likely attributable to cells since it is present in all cell samples but not the 0% (agar only) sample. This implies that the intermediate and long components are associated with the agar. The long T_2 value decreased similar to the long T_2 of the BE analysis. The intermediate T_2 value decreased also, and the magnitude was similar to the short component of the BE analysis. Likewise, the intermediate T_2 fraction from the NNLS analysis decreased in a similar manner as the short fraction of the BE analysis. These results suggested that the intermediate T_2 of the NNLS analysis and the short T_2 of the BE analysis were measuring the same agarose component in the samples. Further, this short agarose component dominated the monoexponential T_2 correlation with cell density.

For future studies we plan to further investigate the agarose components by changing the properties that affect T_2 such as agarose concentration and temperature . We will also investigate how shrinking/swelling the cells affects the presumed cellular component .

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