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Towards Early Glioblastoma Detection:

In Vivo MR Imaging and Spin Dynamics Simulations

A dissertation submitted in partial satisfaction of the

requirements for the degree Master of Science

in Chemistry

by

Guan Wang

2019

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ABSTRACT OF THE DISSERTATION

Towards Early Glioblastoma Detection:

In Vivo MR Imaging and Spin Dynamics Simulations

by

Guan Wang

Master of Science in Chemistry

University of California, Los Angeles, 2019

Professor Louis Bouchard, Chair

Early detection of high-grade malignancy, such as glioblastoma (GBM), using new contrast mechanism and enhanced magnetic resonance imaging (MRI) techniques increases the treatment options available, and therefore may expand survival in GBM beyond the natural history of the disease and possibly increase the patients' survival rate. To achieve early detection, it is important to evaluate commonly used MRI techniques, as well as to develop a theoretical model of GBM growth to predict the consequential changes in MR parameters and to evaluate the performance of newly developed MRI techniques. For this purpose, popular MRI methods such as the spin-echo, the Carr-Purcell-Meiboom-Gill (CPMG), and the spin-locking radio-

frequency (RF) pulse sequences were used to acquire *in vivo* T₂-weighted and T₁ρ-weighted MR images of orthotopic GBM mouse/rat models from U87MG and C6 cell lines. Statistical results (N = 18) showed that, while the spin-echo T₂-weighted MR imaging may not provide the required contrast to detect early GBM, the CPMG T₂-weighted MR imaging and the spin-locking T₁ρ-weighted MR imaging did slightly improve the early GBM contrast, but at the cost of significantly elevated specific absorption rate (SAR) and increased potential risk, if applied to human patients. To facilitate the development of innovative MRI RF pulse sequences to enhance early GBM contrast with low SAR, a simple, computationally efficient theoretical model on early GBM was proposed based on the experimental observation that vessel density decreases together with the increase of vessel size during the early stage of the GBM growth. Based on this model, Monte Carlo spin dynamics simulations were carried out by solving the Bloch equations for the water magnetizations diffusing in the magnetic-field gradients induced by paramagnetic deoxyhemoglobin in the vessels. The interplay among the spin dynamics, early GBM contrast, RF pulse sequences, vessel distributions, and GBM staging can be numerically evaluated. The simulated early GBM contrast and the relaxation time constants, T₂ and T₁ρ, from the spin-echo, the CPMG, and the spin-locking RF pulse sequences were compared with those from the *in vivo* MR imaging of orthotopic early GBM mouse models to study the relaxation mechanisms. It may also serve as a computationally efficient alternative for laboratory use of animals following the 3R (reduction, refinement and replacement) strategy in evaluating the performance of commonly use or newly developed MR methods for early GBM detection.

The dissertation of Guan Wang is approved.

William M Gelbart

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Louis Bouchard, Committee Chair

University of California, Los Angeles

2019

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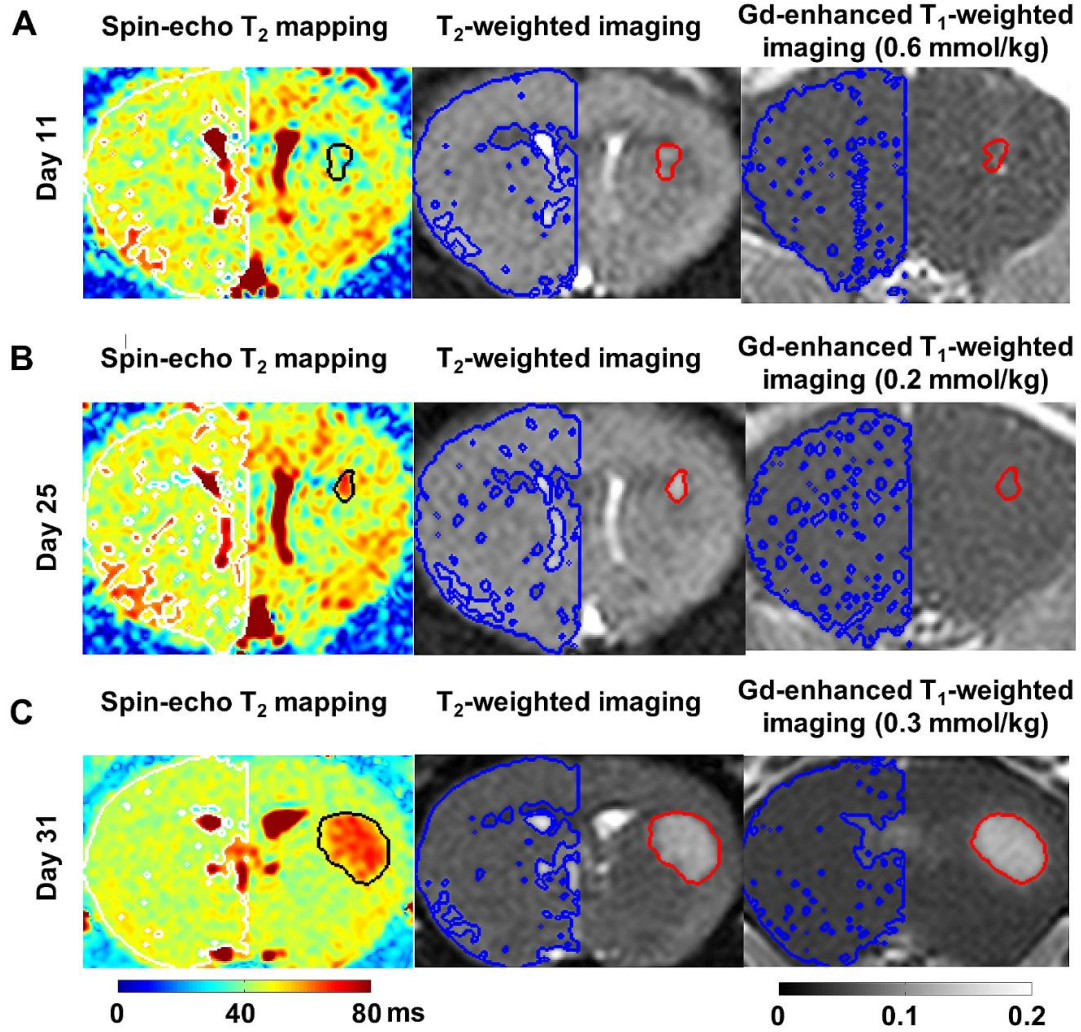


Figure 1. Development of GBM contrast in one orthotopic U87MG mouse model during its clinical course, as acquired by popular MRI pulse sequences. For late-stage GBM (*e.g.*, 30 days after tumor implantation), the metabolic by-products from the GBM cells' mitosis and growth increase the local osmotic gradient of the extracellular fluid. This results in the ingress of fluid from the intravascular space to increase the T_2 time constant and decrease the T_1 time constant to make GBM detectable in the spin-echo T_2 -weighted images and the gadolinium-enhanced inversion-recovery T_1 -weighted images, respectively.

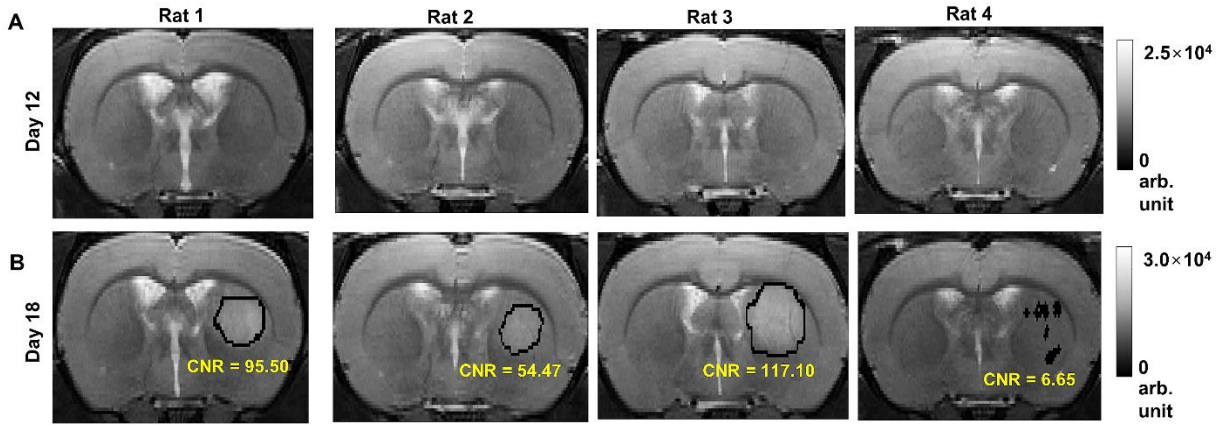


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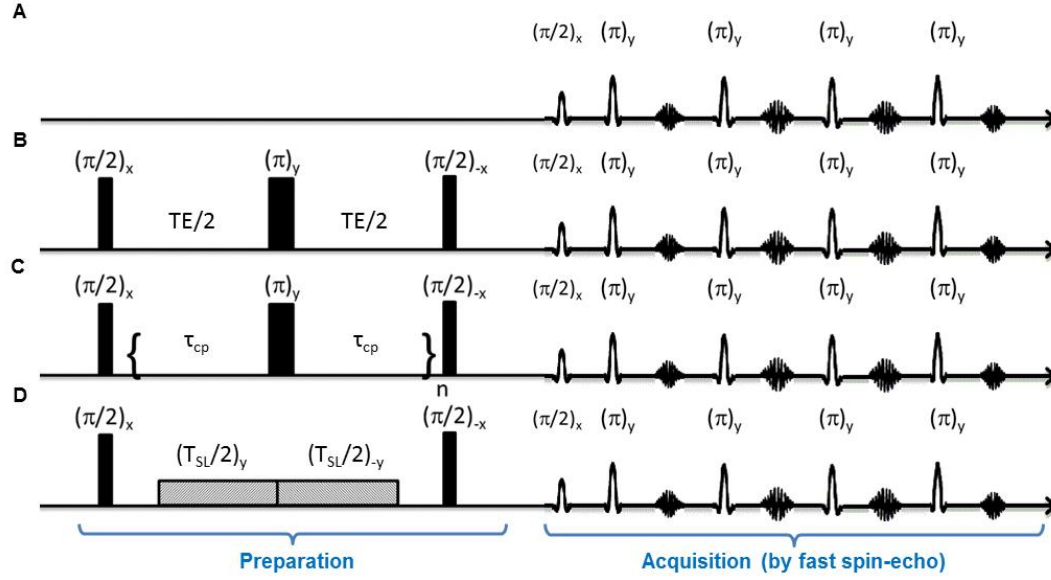


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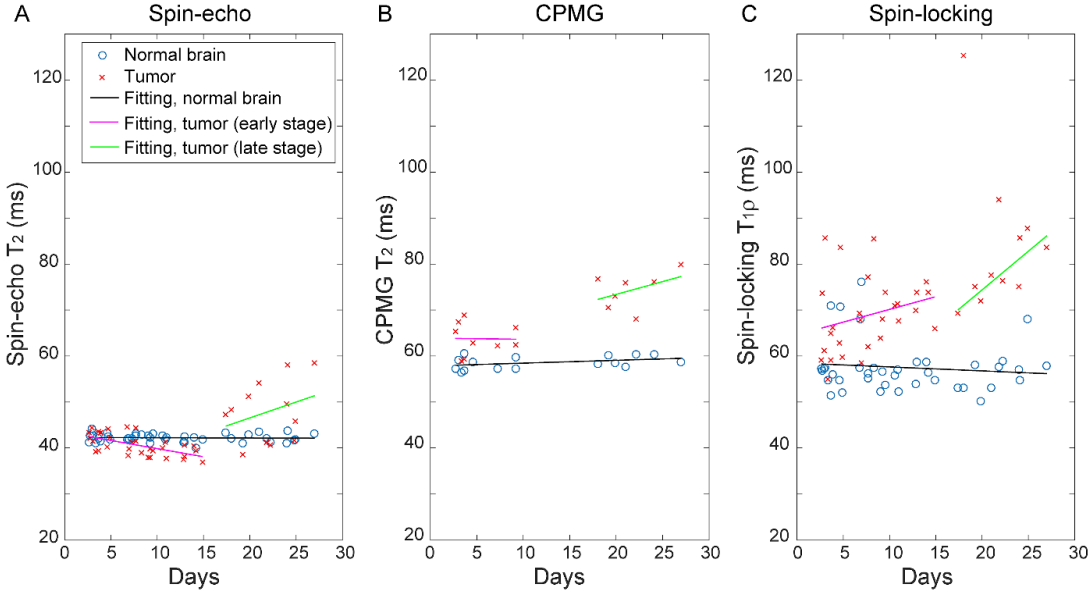


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The linear regressions of the normal brain tissue relaxation time constants are (in ms): $T_{2,SE}^{brain} = -5.47 \times 10^{-3} \times \text{days} + 42.30$, $T_{2,CPMG}^{brain} = 6.12 \times 10^{-2} \times \text{days} + 57.85$, and $T_{1\rho,SL}^{brain} = -8.62 \times 10^{-2} \times \text{days} + 58.50$, respectively. The linear regressions of the early-stage GBM (*i.e.*, within 15 days after tumor implantation) relaxation time constants are (in ms): $T_{2,SE}^{GBM} = -0.35 \times \text{days} + 43.41$, $T_{2,CPMG}^{GBM} = -0.025 \times \text{days} + 63.89$, and $T_{1\rho,SL}^{GBM} = 0.55 \times \text{days} + 64.60$, respectively. The linear regression of the late-stage GBM (*i.e.*, more than 15 days after tumor implantation) relaxation time constants are (in ms): $T_{2,SE}^{GBM} = 0.69 \times \text{days} + 32.77$, $T_{2,CPMG}^{GBM} = 0.56 \times \text{days} + 62.28$, $T_{1\rho,SL}^{GBM} = 1.68 \times \text{days} + 40.80$.

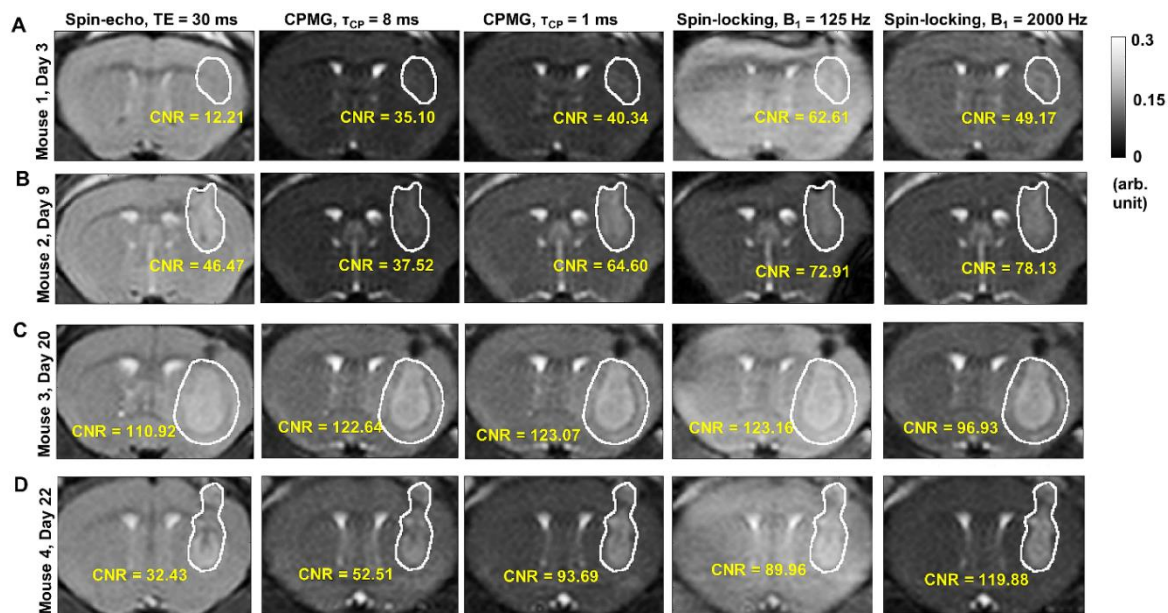


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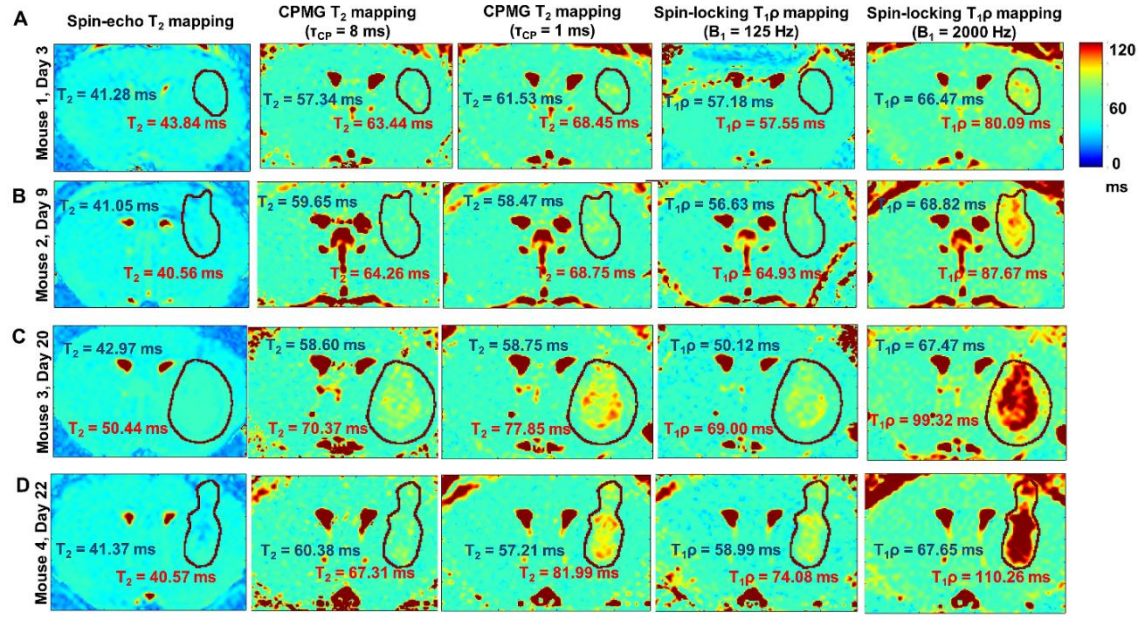


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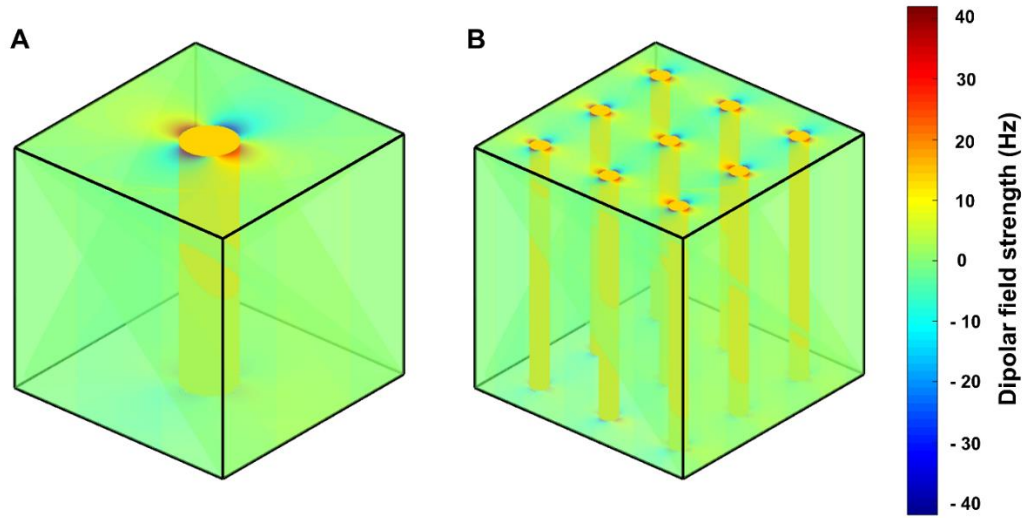


Figure 7. 3-D magnetic field distribution for a voxel with (A) 1 vessel-cylinder and (B) 9 vessel-cylinders. The color map shows the z-component of the magnetic field (B_z) generated by vessel cylinder(s) with blood volume fraction $Bvf = 0.04$. Outside the vessel, as described in Equation 2, B_z rapidly decreases with the distance from the vessel cylinder.

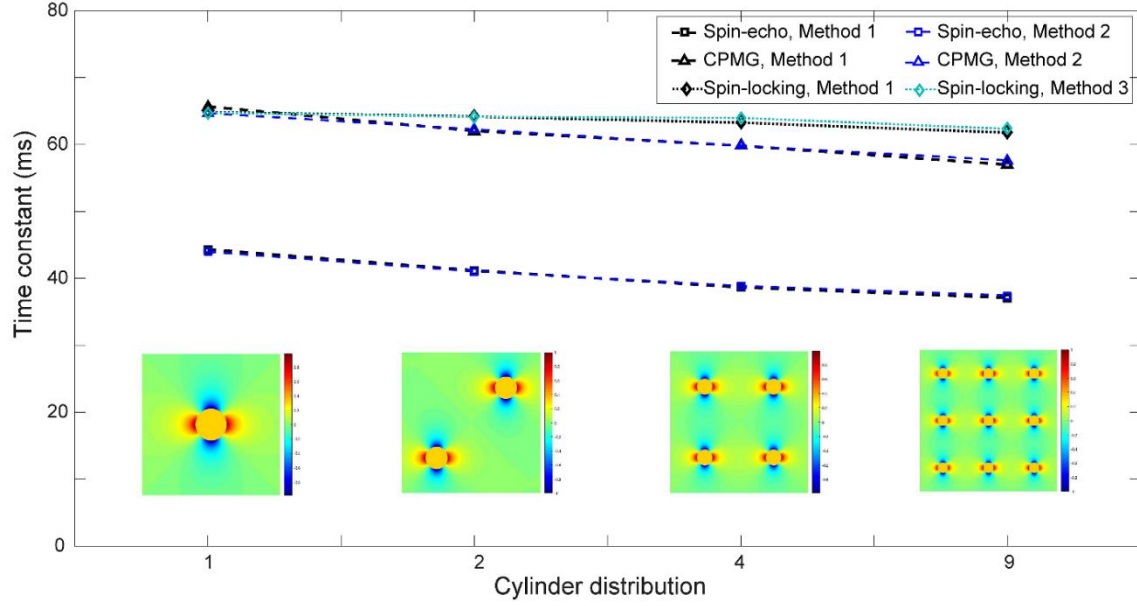


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1. Introduction

Glioblastoma (also called glioblastoma multiforme, GBM) is one of the most challenging diseases to treat in clinical oncology due to its high mortality rates and inefficient conventional treatment methods [1]. Difficulties with early detection, intra-tumor heterogeneity [2-4], post-surgical recurrences [5], and resistance to chemotherapy and radiotherapy [6] are important reasons for the poor prognosis of those with GBM. Many studies have shown that current therapies, including surgery, chemotherapy, radiotherapy, gene therapy [7], and immunotherapy [8, 9], extended patient survival in GBM beyond that allowed by the natural course of the disease. Despite the development of treatment options, the 5-year survival rate reported most recently is only 5.6% [10]. In the vast majority of cases, GBMs are ultimately incurable, as they are typically detected far too late in their clinical course, as illustrated in Figure 1 and Figure 2 using orthotopic U87MG mouse models and C6 rat models, respectively. For late-stage GBM, the metabolic by-products from the GBM cells' mitosis and growth increase the local osmotic gradient of the extracellular fluid. This results in the ingress of fluid from the intravascular space to increase the magnetic resonance (MR) T_2 time constant and decrease the MR T_1 time constant, making GBM detectable in the spin-echo T_2 -weighted MR images and the inversion-recovery T_1 -weighted MR images, respectively. Unfortunately, by the time GBM becomes symptomatic, it is almost always too late in its biological course such that isolated tumor/stem cells would have migrated far beyond the MR imaging-defined tumor mass. These motile cells will ultimately establish additional tumor nidus in the margin of the resection, at some distance away from the margin, or even in the opposite hemisphere. At this time, no effective prevention or early MR imaging for GBM is available.

Early detection of cancer may improve survival rate and decrease morbidity. This is of great importance for high-grade malignancy such as GBM. The goals for early cancer detection is to find them when they are small, find them when they are local, find them before they turn malignant, and find them when they may still be curable by some minimally invasive surgical method or even by stereotactic radiation methods such as brachytherapy or radiosurgery. Many studies have shown that current therapies (surgery, radiation, chemotherapy, *etc.*) expand survival in high-grade malignancy beyond the natural history of the disease [11]. For example, according to a study conducted by Yabroff *et al.* [12], for adult GBM patients treated with both chemotherapy and radiotherapy, the median survival was approximately 15 months; for patients who received either chemotherapy or radiotherapy, the median survival dropped to 7 months; for patients who did not receive any standard treatment, the median survival was only 2 months. Moreover, Ohgaki *et al.* [13] reviewed population-based studies on survival rates in astrocytic and oligodendroglial gliomas, and found that the median survival time for patients diagnosed with astrocytoma that was still at low-grade was 5.6 years, but for patients diagnosed with astrocytoma that has progressed from low-grade glioma to the high-grade GBM was only 4.9 months. Consequently, early detection of high-grade malignancy, such as glioblastoma (GBM), using new contrast mechanism and enhanced MRI techniques increases the treatment options available, and therefore may expand survival in GBM beyond the natural history of the disease and possibly increase the patients' survival rate.

In this work, we first presented *in vivo* T₂-weighted and T₁ρ-weighted MR images of orthotopic GBM mouse models infected with human U87MG cell line and orthotopic GBM rat models infected with C6 cell line, respectively. Two cell lines were used in this work to confirm the scientific validity and general applicability of our study and conclusion. Since tumor models

created by different cell lines may generate differences in evolution and MR parameters, it is important to study different experimental tumor models in *in vivo* and preclinical trials. We then presented *in vivo* MR images of the above-mentioned orthotopic GBM mouse/rat models acquired by the commonly used spin-echo, Carr-Purcell-Meiboom-Gill (CPMG), and spin-locking RF pulse sequences. Statistical results ($N = 18$) showed that spin-echo T_2 -weighted MR imaging may not provide the required contrast to detect early GBM. The CPMG T_2 -weighted MR imaging and the spin-locking $T_{1\rho}$ -weighted MR imaging could slightly improve early GBM contrast, but at the cost of elevated specific absorption rate (SAR) and increased potential risk to the patients. This is not suitable to detect early-stage diseases, which requires a proven, safe, and acceptable screening technique.

Building a realistic and flexible biophysical model for early GBM is important, not only to accelerate the development of innovative MRI pulse sequences for early GBM detection with low SAR, but also to provide an alternative for laboratory use of animals following the 3R (reduction, refinement, and replacement) strategy. Over the past two decades, several analytical methods of functional MRI (fMRI), such as cerebral blood volume mapping (CBV), positron emission tomography (PET), and blood oxygenation level-dependent (BOLD) imaging, have been studied, in which fMRI uses intrinsic signal-change of NMR to develop and enhance the contrast originated from magnetic susceptibility. Most of the current papers on BOLD signal in the literature focus on improving the models, and yet, few of them describe the relationship with realistic early GBM relaxation. Thus we propose the first model for early GBM and normal brain tissue inspired by Ogawa *et al.* [14], in which the relationship between the transverse relaxation, pulse sequences used for imaging, and vessel distributions can be evaluated.

Through *in vivo* and *ex vivo* study on rat model of C6 glioma, Valable *et al.* [15] found that the blood volume fraction (BVf) in the tumor center and periphery remained nearly unchanged until day 20, while the vessel size index in the tumor kept increasing until day 25 and became significantly higher than that in the contralateral striatum on day 11. Combining these experimental observations that BVf remained about the same while the average vessel size increased with the GBM growth and the BOLD model, we proposed a simple, computationally efficient theoretical model for MRI studies on early GBM. Using the proposed model, Monte Carlo spin dynamics simulations were carried out by solving the Bloch equations for the water magnetizations diffusing in the magnetic-field gradients induced by paramagnetic deoxyhemoglobin in the vessels. The interplay among the spin dynamics, GBM contrast, RF pulse sequences used for imaging, vessel distributions, and GBM staging can be numerically evaluated. The simulated early GBM contrast and relaxation time constants, T_2 and $T_{1\rho}$, from the spin-echo, the CPMG, and the spin-locking RF pulse sequences were then compared with those from *in vivo* MR imaging of orthotopic early GBM mouse models to study the relaxation mechanisms. To the best of our knowledge, the proposed work is the first GBM computational model based on the previous pathological finding of increase in vessel size, while maintaining similar blood vessel volume fraction. It allows semi-quantitative and qualitative comparison with *in vivo* MR images and provide a simple, computationally efficient alternative in evaluating the performance of commonly use or newly developed MR methods for early GBM detection.

2. Results

2.1. *In Vivo* MR Imaging

To evaluate the feasibility of using existing popular MRI RF pulse sequences for early GBM detection, a GBM mouse model ($N = 18$) was established using an intracranial inoculation of U87MG cells. The imaging studies were performed from Day 3 through Day 28 following tumor inoculation. A Varian INOVA 7-T high-resolution NMR spectrometer equipped with a 30-mm I.D. Varian Millipede micro-imaging probe was used for the investigations. The RF pulse sequences used in this work are shown in Figure 3. Since this micro-imaging system has superior static and RF field homogeneity, and accurate RF flip angle calibration, the choice of the spin-echo (Figure 3B), the CPMG (Figure 3C), and the spin-locking (Figure 3D) pulse sequences to prepare the spin system to high-contrast states (then acquired by fast spin-echo) is mainly to compare their sensitivity in detecting early GBM by various rephasing mechanisms.

Figure 4 shows the relaxation time constants, T_2 and $T_{1\rho}$, of the GBM and the normal brain tissue, as acquired by the spin-echo (SE, Figure 3B), the CPMG (Figure 3C, $\tau_{CP} = 8$ ms), and the spin-locking (SL, Figure 3D, $B_1 = 125$ Hz) RF pulse sequences, respectively. The observed T_2 time constant by the spin-locking pulse sequence is commonly referred as $T_{1\rho}$ [16, 17]. The linear regressions of the normal brain tissue relaxation time constants are (in ms):

$$T_{2,SE}^{\text{brain}} = -5.47 \times 10^{-3} \times \text{days} + 42.30, T_{2,CPMG}^{\text{brain}} = 6.12 \times 10^{-2} \times \text{days} + 57.85, \text{ and}$$

$$T_{1\rho,SL}^{\text{brain}} = -8.62 \times 10^{-2} \times \text{days} + 58.50, \text{ respectively. It is clear that the relaxation time}$$

constants of the normal brain tissue remain constant over the course of the GBM growth, regardless the MR techniques used. For example, the average T_2 time constant of the normal brain tissue for the most commonly used MR pulse sequence, the spin-echo pulse sequence

(Figure 3B), is $T_{2,SE}^{\text{brain}} = 42.24$ ms, with a standard deviation 0.88 ms. On the other hand, the linear regression of the GBM T_2 time constants over the course of the GBM growth from Day 3 to Day 28 is found to be (in ms) $T_{2,SE}^{\text{GBM}} = 0.35 \times \text{days} + 38.51$, consistent with the experimental observation that GBM generates brighter T_2 contrast as it grows. To distinguish early-stage GBM, we define time constant threshold for hyperintensity as one standard deviation higher than the average T_2 of the normal brain, which is found to be 15 days after tumor implantation. The linear regressions of early-stage GBM (*i.e.*, earlier than 15 days after tumor implantation) relaxation time constants are (in ms): $T_{2,SE}^{\text{GBM}} = -0.35 \times \text{days} + 43.41$, $T_{2,CPMG}^{\text{GBM}} = -0.025 \times \text{days} + 63.89$, and $T_{1\rho,SL}^{\text{GBM}} = 0.55 \times \text{days} + 64.60$, respectively.

To eliminate the effect from tumor injection to the differences in MR parameters between the GBM and the normal brain tissue, we have also carried out control experiment in which only PBS solution was injected into the mouse brain. The average T_2 time constants obtained from the spin-echo T_2 -weighted images (Figure 3B) of the PBS area and the normal brain tissue were fitted to be (T_2 for PBS area was listed first): 42.1 ms and 42 ms (Day 1); 41 ms and 41.8 ms (Day 2); 36 ms and 41.1 ms (Day 3); 39.4 ms and 40.7 ms (Day 6.5); 39.9 ms and 42.2 ms (Day 8); 40.1 ms and 41.7 ms (Day 19). The results show that the damage to the brain by the needle and the insertion of a certain volume of fluid do not contribute significantly to the difference in the MR parameters between the GBM and the normal brain tissue, and the difference we observed can be attributed to the GBM and its growth.

For visual comparison, Figure 5 and Figure 6 show representative T_2 -weighted and $T_{1\rho}$ -weighted MR images and time-constant mappings from four different mice, acquired by the spin-echo (Figure 3B), the CPMG (Figure 3C), and the spin-locking (Figure 3D) RF pulse sequences

on Day 3, Day 9, Day 20, and Day 22, respectively. For each of CPMG and spin-locking, two sets of experimental parameters were used: $\tau_{CP} = 8$ ms and 1 ms (half of CPMG inter-pulse spacing), and $B_1 = 125$ Hz and 2000 Hz (spin-locking field strength), to investigate how RF power affect the detection sensitivity. For the spin-echo pulse sequence, the nearly indistinguishable T_2 parameters (Figure 4A) and a visual inspection on Figure 5 and Figure 6 (first column) suggest that it may not provide the required contrast to detect early GBM. On the other hand, the CPMG T_2 -weighted MR imaging with short echo spacing (*e.g.*, $\tau_{CP} = 1$ ms) and the spin-locking $T_1\rho$ -weighted MR imaging with high locking-field (*e.g.*, $B_1 = 2000$ Hz) slightly improve early GBM contrast. For late-stage GBM (more than 15 days after tumor implantation), contrasts are enhanced greatly for all pulse sequences as edema evolves and the local magnetic environment of the GBM becomes drastically different from that of the normal brain tissue.

2.2. Spin Dynamics Simulations

Monte Carlo spin dynamics simulations were carried out by solving the Bloch equations for the water magnetizations diffusing in the magnetic-field gradients induced by paramagnetic deoxyhemoglobin in the vessels, as described in details in sections 4.5-4.9. The interplay among the spin dynamics, GBM contrast, RF pulse sequences used for imaging, vessel distributions, and GBM staging can be numerically evaluated. As mentioned in the Introduction, based on the experimental observation by Valable *et al.* [15] that BVf remained about the same while the average vessel size increased with the GBM growth (until very late stage), 1 vessel-cylinder and 9 vessel-cylinders with the same BVf were chosen to represent the later stage and the earlier stage of the GBM growth, respectively. Using the form of the magnetic field given by Equation

2, 3D plots of the magnetic field created by 1 vessel-cylinder and 9 vessel-cylinders, respectively, are shown in Figure 7. The color map shows the z-component of the magnetic field (B_z) generated by vessel-cylinder(s) with the same $B_{vf} = 0.04$. For both cases, it is clear that water magnetizations experience a rapidly decreasing B_z with distance from the vessel-cylinder when they are moving outside the vessel. The differences in the magnetic field distribution created by 1 vessel-cylinder and 9 vessel-cylinders are further discussed in Figure 8. While the field distribution of B_z for 9-vessel-cylinder voxel and 1-vessel-cylinder voxel is almost the same (Figure 8A), the change in B_z per random walk step for a voxel with 9 vessel-cylinders shows a broader distribution than that for a voxel with 1 vessel-cylinder (Figure 8B).

The relation between vessel cylinder distribution and T_2 and $T_{1\rho}$ time constants is summarized in Figure 9. The figures inserted above the number of vessel-cylinder(s) are the corresponding magnetic field distributions. Fewer, larger vessel cylinders represent the later stage of the GBM growth. Vessel-cylinders are distributed such that the distance between nearest pairs remains constant across voxels when aligned periodically. Vessel-cylinder radius varies with the number of vessel cylinders from 9×10^{-6} m to 3×10^{-6} m to maintain constant $B_{vf} = 0.04$ for the four cases. Background T_2 and $T_{1\rho}$ relaxation parameters in the Bloch equation are chosen by comparing with experimental results shown in Figure 4. The background T_2 and $T_{1\rho}$ relaxation parameters used for spin echo, CPMG, and spin locking are 55 ms, 70 ms, and 65 ms, respectively. As mentioned in sections 4.6-4.9, for each pulse sequence used (spin-echo, CPMG, and spin-locking), two simulation methods were used to prove accuracy and provide computational efficiency. It is clearly shown in Figure 9 that the results from the two methods converge to within 1.5%. Agreement between different methods indicates that simulations are consistent in our model.

3. Discussion

3.1. Relaxation Mechanism

The dephasing mechanisms for water magnetizations diffusing among magnetic nanoparticles in a system have been extensively studied in the last two decades [18]. In the absence of a refocusing pulse, the dephasing mechanisms of the water magnetizations can be grouped into two regimes, depending on the size of the magnetic nanoparticles: the motional averaging regime (small particle sizes) and the static dephasing regime (large particle sizes). Static dephasing regime was originally proposed to describe the magnetic nanoparticles with smaller magnetic field gradient and large inter-particle distance compared to the diffusion distance experienced by the water magnetizations nearby. Subsequently, the resulting relaxation rate will not be affected by the random walk of the water magnetization and will approach a static limit. For large magnetic particles in the static dephasing regime, phase dispersion of surrounding water magnetizations may be partially refocused using spin echo techniques, which is referred to as the slow motion regime. In this work, we extend the dephasing mechanism induced by large magnetic nanoparticles under refocusing pulses (*i.e.*, the slow motion regime) to the dephasing of the water magnetizations in our vessel-cylinder model.

A refocusing RF pulse is a 180° pulse that is applied at time $TE/2$ (in the spin-echo pulse sequence) or τ_{CP} (in the CPMG pulse sequence) after the initial excitation pulse. During diffusion, water magnetizations experience magnetic field gradients, which in turn dephase the ensemble by letting water magnetizations precess at different precession frequencies. When the ensemble of water magnetizations is partially rephased by the 180° pulses, the phases are reversed back in time to some extent by reversing the order of the fast and the slow components,

and dephasing is partially diminished along this process. Therefore, the fast decay between $t = 0$ and $t = \tau_{CP}$ will be partially recovered after the 180° pulse, and the signal after τ_{CP} increases and peaks at $TE = 2 \tau_{CP}$, which is referred to as the "echo" of the original signal. Fitting the exponential decay function over these echo amplitudes results in a decay much slower than the decay between echoes and the resulting decay rate, R_2 , is significantly lower than the cases when there is no refocusing 180° pulse.

In the presence of refocusing 180° pulses, the relaxation depends on the extent of rephasing. The refocusing is more effective when more water magnetizations are localized to a consistent local magnetic field. Therefore, the longer time water magnetizations can freely evolve under a varying field, the more dephasing occurs, *i.e.*, shorter pulse spacing yields lower R_2 rates (longer T_2 relaxation time) than longer ones. An analogy can be established between CPMG and spin-locking in that spin-locking operates with consecutive rephasing pulses at an even shorter τ_{CP} in this experiment. The frequent refocusing in spin-locking makes the difference in field distributions most pronounced and in turn generates the best contrast [16, 17]. Our *in vivo* experimental results and simulation results suggest that the relaxation mechanism in the GBM and the normal brain tissue, in which the blood vessels generate local magnetic field gradients and lead to the loss of phase coherence among the water magnetizations nearby, can be understood by the slow motion regime. Experimental results from Figure 5 and Figure 6 show that the CPMG and the spin-locking pulse sequences suppress the relaxation for both GBM (at any stage) and normal brain tissue, compared to the spin-echo pulse sequence, and the shorter the inter-pulse spacing (or the stronger the B_1 locking field), the more the relaxation is suppressed. This trend is confirmed by the computer spin dynamics simulation results shown in Figure 9, where we simulated 1, 2, 4, and 9 vessel-cylinders to mimic normal brain tissue and different

stages of the GBM growth. Such observations are consistent with the relaxation scheme in the slow motion regime described above.

3.2. SAR Concerns

Although the CPMG T_2 -weighted MR imaging with short inter-pulse spacing (*e.g.*, $\tau_{CP} = 1$ ms) and the spin-locking $T_{1\rho}$ -weighted MR imaging with high locking-field (*e.g.*, $B_1 = 2000$ Hz) slightly improve early GBM contrast, as shown in Figure 5 and Figure 6, it is at the cost of elevated specific absorption rate (SAR) and increased potential risk to the patient [19]. In the clinical MRI scans on brain tumor patients, the inter-pulse spacing chosen in the CPMG pulse sequence is usually longer than 7 ms [20-22]. Therefore, the short inter-pulse spacing CPMG and high locking-field spin-locking pulse sequences that generated improved contrast in the presented work, if applied to human patients, may raise potential risk due to elevated SAR. In addition, special attention on SAR needs to be paid for GBM patients, as previous study showed that peak local SAR estimation using a healthy patient model may be lower than the true peak local SAR in a brain tumor patient [23].

3.3. Vessel Density Dependence

As shown in Figure 7, the strength of the induced magnetic field gradients depends on spatial locations. Water magnetizations experience the strongest magnetic field gradients when they diffuse around the vessel-cylinders. As mentioned previously, T_2 relaxation in the slow motion regime depends on the extent of refocusing. Similar to how more frequent refocusing

pulses suppress the T_2 relaxation, the more the water magnetizations are localized in a relatively homogeneous magnetic-field area, the more effective the refocusing is. Therefore, the refocusing is more effective for water magnetizations surrounding large vessels than those surrounding small vessels where the water magnetizations diffuse to a different magnetic environment more easily. In the 1-vessel-cylinder case where the radius is the largest, magnetic field gradient is weaker compared to the multiple-vessel-cylinder case, as indicated in Figure 8. Thus water magnetizations in the 1-vessel-cylinder voxel would need to diffuse the most steps in order to experience large gradient. For the 9-vessel-cylinder case, the dipolar field fluctuates strongly near each vessel cylinder, and water magnetizations only need to diffuse a few steps to experience a significant change in the magnetic field. The change in T_2 relaxation per cylinder, $\frac{\Delta T_2}{\Delta C}$, decreases as the density of cylinder increases for all pulse sequences, where ΔC is the change in the number of vessel cylinders. This diminution in the effect of vessel density variation can be attributed to the decreasing difference in vessel cylinder radius as discussed in Boxerman *et al.* [24, 25].

3.4. Validity and Accuracy of the Model

Rigorous theoretical modeling on GBM growth is complex, as there are many pathological changes with GBM growth that may affect the MR observables and spin dynamics. To provide a simple and computationally efficient theoretical model for future studies, especially for the development of much more sensitive MRI pulse sequences and imaging modalities for early GBM detection, in this study we focused on the major difference between the early and the late stages of the GBM growth, *i.e.*, the increase in vessel size. The differences in vascular

permeability, blood volume fraction, and magnetic susceptibility between the normal brain tissue and GBM were assumed to be negligible in the early-stage GBM. Vessel network orientations may take important role on induced magnetic field; however, the effect may not be as important in the study of very early stage of GBM formation, due to the small voxel-size in this case, compared to the previous study by Martindale *et al.* [26].

The transverse (T_2) relaxation of the average magnetization of the spin ensemble mainly originates from the fluctuating local magnetic fields experienced by the water protons, due to, for example, diffusion in macroscopic and microscopic magnetic field gradients, magnetization transfer, and spin-spin "flip-flop". In computer spin dynamics simulations, we focus on the dephasing due to the water magnetizations diffusing in the magnetic-field gradients induced by paramagnetic deoxyhemoglobin in the vessel-cylinders and collectively describe the other mechanisms by a phenomenological background relaxation to provide a simple and computational efficient theoretical model. Consequently, the GBM contrast observed in the spin-echo T_2 -weighted images, the CPMG T_2 -weighted images, and the spin-locking $T_{1\rho}$ -weighted images originate not only from the difference in the magnetic field gradients from the vessel-cylinders, but may also from the background relaxation mechanisms. Among various background relaxation mechanisms, the contribution from the T_1 relaxation process should be insignificant, due to the long T_1 time constants in biological tissues (> 1 s) and the similar T_1 time constants between the GBM and the normal brain tissues.

On the other hand, magnetization transfer (MT) is likely to contribute to the observed GBM contrast. MT describes the interactions between "free" water protons and "restricted" water protons that are bound to proteins or other macromolecules. MT can occur either by direct chemical exchange or by indirect dipole-mediated cross relaxation between macromolecular

protons and water protons -- both processes are known to be important in biomedical tissue [27-34]. Selective saturation of the characteristically short T_2 macromolecular proton pool can produce contrast called magnetization transfer contrast, based on the exchange or cross-relaxation process. MT contrast has been shown to be positively correlated with tumor grade and cellularity [31-34]. Selective saturation can be achieved with continuous wave irradiation several kHz off resonance or short, intense δ -pulses on resonance. Therefore, the spin-echo, the CPMG, and the spin-locking pulse sequences used in this work to prepare the spin systems to high-contrast states may accidentally generate MT-weighted imaging. In particular, spin-locking can easily produce MT contrast [35] and CPMG is equivalent to spin-locking in the limit of short inter-pulse spacing, τ_{CP} [36].

Our model can be further refined by incorporating more detailed information and complicated dynamics. For example, more accurate early GBM models that take into account other effects, such as the vessels becoming more disorganized and randomly oriented when GBM grows, changes in the diffusion coefficient of water magnetizations, the increase of vascularization in the periphery, and the MT effect, are under development in our lab now.

4. Materials and Methods

4.1. Orthotopic GBM Mouse Model by U87MG Cell Line

Six-week-old male NOD CB17-Prkd^{scid}/IcrCr1B1tw (NOD/SCID) mice were obtained from BioLASCO Experimental Animal Center (BioLASCO, Taiwan) and bred in a specific pathogen-free room in the animal facility. All animal procedures were in accordance with the regulations approved by the Institution Animal Care and Utilization Committee (IACUC) at National Taiwan University (project identification number: NTU-103-EL-61; date of approval: 10/2014). All operations were performed under anesthesia and all possible effort has been made to minimize pains/suffering of the mice. The U87MG cell line used was purchased from Bioresource Collection and Research Center (BCRC, Taiwan), which was derived from American Type Culture Collection (ATCC). U87MG cell line was chosen because it is one of the most commonly used cell lines in human glioma research. Although a recent study conducted by researchers from the laboratory in which U87MG cell line was established found that the commercially available version of U87MG (from the ATCC) not identical to its patient of origin, U87MG is still believed to be a *bona fide* human glioblastoma cell line of unknown patient origin [37]. It is likely a glioblastoma of central nervous system (CNS) origin.

For tumor implantation, each NOD/SCID mouse was anesthetized with Ketalar (40 mg/kg) and Rompun (15 mg/kg) and placed in a stereotactic frame for accurate location of implantation. U87MG cells (5×10^5 cells, 5 μ L per mouse) were orthotopically inoculated into the center of caudate putamen of the mouse (2.5 mm to the right of bregma, and depth = 3.0 mm). During the imaging acquisition, vital signs of the mouse under anesthesia (about 5% isoflurane

induction, 2% for maintenance in air) was monitored. The flow rate of isoflurane was carefully adjusted to maintain stable heart rate and respiratory rate.

A total of 30 mice were used in this study. One mouse died in the GBM implantation surgery, one mouse was bored a large hole during the surgery, one mouse did not grow GBM, 5 mice encountered hydrocephalus (caused by U87MG cells being injected into lateral ventricle), and the GBM implantation was considered not successful in 4 mice for other reasons. Thus the mice *in vivo* results shown in this paper were obtained from the other 18 mice. Each mouse was measured every 4-7 days between Day 3 and Day 30 to make sure that they have sufficient rest between MR measurements and to fit into the limited MRI machine time available. Nine (9) mice were measured throughout the whole time period, while the other 9 were measured in early stage of the GBM growth only due to death or sacrifice (when GBM became too big).

4.2. Orthotopic GBM Rat Model by C6 Cell Line

9-week-old male Sprague-Dawley and Wistar rats were obtained from National Laboratory Animal Center of Taiwan and bred in a specific pathogen-free room in the animal facility. No significant difference in tumor growth rate and MR results was observed between these two strains used in the rat experiment. All animal procedures were in accordance with the regulations approved by the Institution Animal Care and Utilization Committee (IACUC) at the Institute of Biomedical Sciences, Academia Sinica (protocol ID: 17-02-1050). All operations were performed under anesthesia and all possible effort has been made to minimize pains/suffering of the rats. The C6 cell line used was purchased from Bioresource Collection and Research Center (BCRC, Taiwan), which was derived from American Type Culture Collection

(ATCC). For tumor implantation, each rat was placed in a stereotactic frame for accurate location of implantation. C6 cells (0.9×10^5 cells, 0.6 μL per rat) were orthotopically inoculated into the striatum of the rat (Bregma = 0.2 mm, lateral = 3.0 mm, and depth = 5.0 mm) using a 30-gauge needle (Hamilton, NV, USA). The GBM implantation in all 4 rats was successful. MR images on all 4 rats were acquired 12 and 18 days after the GBM implantation.

4.3. *In Vivo* MR Imaging Acquisition

All *in vivo* mouse MR images were acquired by a Varian INOVA 7-T NMR spectrometer (Varian Inc., USA) equipped with a 30-mm I.D. Varian Millipede micro-imaging probe and self-shielded gradient systems with a maximum strength of 100 G cm^{-1} in each direction (Resonance Research Inc., USA). Images were prepared by the spin-echo (Figure 3B), the CPMG (Figure 3C), and the spin-locking (Figure 3D) pulse sequences to high contrast states and then acquired by the fast spin-echo (FSE) pulse sequence (Figure 3A) with repetition time (TR) = 7.5 s, number of scans (NS) = 1, echo spacing = 10 ms, number of echos = 8, field of view (FOV) = 2.56 cm $\times$ 2.56 cm, matrix size = 128 $\times$ 128, zero padding = 512 $\times$ 512, and slice thickness = 0.8 mm. For the spin-echo pulse sequence (Figure 3B), echo time (TE) = 10, 30, 50, 70, and 90 ms, respectively. For the CPMG pulse sequence (Figure 3C), τ_{CP} = 8 ms, 1 ms, and number of 180° pulses (n) = 2, 4, 8, 16, 32, respectively, where τ_{CP} is half of the interval between successive 180° pulses in a CPMG sequence ($\tau_{\text{CP}} = \text{TE}/2$). For the spin-locking pulse sequence (Figure 3D), locking field B_1 = 125 Hz, 2000 Hz, and T_{SL} = 10, 30, 50, 70, 100 ms, respectively.

All *in vivo* rat MR images were acquired by horizontal 7.0-T spectrometer (PharmaScan 70/16, Bruker, Germany) equipped with a 372-mm birdcage transmitter coil and a separate

quadratic surface coil for signal detection and active shielding gradient of 300 mT/m in 80 μ s.

Images were acquired by the fast spin echo pulse sequence (Figure 3A) with TR = 3 s,

TE = 60 ms, FOV = 2.56 cm $\times$ 2.56 cm, matrix size = 256 $\times$ 128, and slice thickness = 1 mm.

4.4. *In Vivo* MR Imaging Analysis

Because the U87MG cells were implanted into the right brain, the mean intensity and standard deviation (STD) of the left side of the bilaterally symmetric brain were calculated. Then the normal brain tissue area was selected from the range of mean $\pm$ 3 $\times$ STD to avoid the extremely high/low intensity signals from certain characteristic regions in the brain, such as lateral ventricle (contains cerebrospinal fluid), corpus callosum, etc., as they have very different MR parameters compared to the rest of the brain.

The region of interest for the GBM was defined by images and mappings acquired by more sensitive MR methods with superior contrast and low SAR developed in our lab [38-44], and the location of the GBM was also confirmed by histopathology slides taken after the animals were sacrificed at late stage.

For all MR images, contrast-to-noise ratios (CNR) is calculated based on:

Equation 1.

$$\text{CNR} = \frac{|S(\text{tumor}) - S(\text{brain})|}{\sigma(\text{noise})}$$

where $S(\text{tumor})$ and $S(\text{brain})$ are the average signal intensities for the tumor (in the right brain) and the normal brain tissue (in the left brain), respectively, and $\sigma(\text{noise})$ is the standard deviation of the image noise.

4.5. Early GBM Simulation Model

To mimic early GBM microenvironment, our simple, computationally efficient theoretical model was based on the experimental observation that vessel density decreases together with the increase of vessel size during the early stage of the GBM growth [15]. It consisted of a voxel cube with parallel infinite vessel-cylinders representing blood vessels distributed uniformly inside. The total volume of the vessel-cylinders was kept constant and the cube volume was determined by the BVf. From [15], BVf was taken as 0.04. The vessel radius, R , was set to be $\frac{9 \times 10^{-6}}{\sqrt{N}}$ m, where N is the number of vessel-cylinders distributed uniformly inside the voxel cube. N decreased during the GBM growth. Each infinite vessel cylinder contributed an induced dipolar field, ω_B , at position $\vec{r}$ along the direction of the externally applied static superconducting magnetic field, B_0 [14]:

Equation 2.

$$\omega_B = \begin{cases} 2\pi\Delta\chi(1-Y)\frac{B_0}{\gamma}\sin^2(\theta)\left(\frac{R}{r}\right)^2\cos(2\varphi), & r > R \\ 2\pi\Delta\chi(1-Y)\frac{B_0}{\gamma}\left(\cos^2(\theta) - \frac{1}{3}\right), & r < R \end{cases}$$

where γ is the ^1H gyromagnetic ratio, $(1 - Y)$ is the degree of deoxygenation of the blood (assumes the value of 0.3 [15]), θ is the angle between the vessel-cylinder orientation and B_0 , R

is the vessel-cylinder radius, r is the shortest distance to the vessel-cylinder center of interest, ϕ is the angle between $\bar{r}$ and the projection of the external static superconducting magnetic field onto the plane perpendicular to the vessel cylinder orientation, and $\Delta\chi$ is the susceptibility difference between entirely deoxygenated blood and entirely oxygenated blood (assumes the value of 0.15 ppm [24]).

4.6. Monte Carlo Spin Dynamics Simulations

To optimize the efficiency and accuracy of the spin dynamics simulations, two different simulation methods were carried out to evolve the water magnetizations under the spin-echo and the CPMG pulse sequences. Initially 4000 water magnetizations were randomly distributed inside the cube for Method 1 and on a plane perpendicular to the vessel-cylinders for Method 2. During the random walk process that mimics the water diffusion, each step was taken as a random vector on a sphere for Method 1 or on a circle for Method 2 with step-size determined by three-dimensional [44] or two-dimensional diffusions, respectively. The transverse magnetization of the diffusing water magnetizations dephased due to local field inhomogeneity induced by paramagnetic deoxyhemoglobin inside blood vessels [45]. The water diffusion coefficient, D , was taken as $1 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ and the time-step, Δt , was taken as 0.05 ms. Small time-steps are required to make sure that water magnetizations sufficiently sample the induced dipolar fields and will not jump over the vessel-cylinders in a few steps. The vessel-cylinders were assumed to be impermeable for water diffusion. Random-walk steps that penetrated vessel-cylinders were repeated until a collision-free step was generated. By assuming the spatial periodicity of voxels, periodic boundary condition was applied. Water magnetizations that

walked out of the voxel re-entered from the opposite side of the voxel cube. To study the effect of vessel-cylinder aggregation, simulations were repeated with uniform distributions of $N = 1, 2, 4$, and 9 vessel cylinders, respectively. Simulation results were averaged over 16 θ angles between 0 and π , weighted by $\sin(\theta)$. All simulations were programmed using Matlab (The Mathworks Inc.).

4.7. Simulation Method 1 – Analytical Method

The Bloch equation describes the classical spin dynamics of a spin magnetization $\mathbf{M} = M_x \mathbf{i} + M_y \mathbf{j} + M_z \mathbf{k}$ in the presence of a magnetic field $\mathbf{B} = B_x \mathbf{i} + B_y \mathbf{j} + B_z \mathbf{k}$, where $\{\mathbf{i}, \mathbf{j}, \mathbf{k}\}$ are the unit vectors of the Cartesian coordinate system. Via a complete analytical solution of the Bloch equation [46], exact time evolution of a spin magnetization $\mathbf{M}(t)$ for each step with duration time Δt during diffusion can be obtained by applying the propagator $\mathbf{U}(\Delta t, 0)$:

Equation 3.

$$\mathbf{M}(\Delta t) = \mathbf{U}(\Delta t, 0) \mathbf{M}(0)$$

$$\mathbf{U}(\Delta t, 0) = e^{-\Gamma \Delta t}$$

$$\Gamma = \begin{bmatrix} \frac{1}{T_2} & -\gamma B_z & \gamma B_y \\ \gamma B_z & \frac{1}{T_2} & -\gamma B_x \\ -\gamma B_y & \gamma B_x & \frac{1}{T_1} \end{bmatrix}$$

where $-\Gamma$ is the matrix form of the system of Bloch equations for the magnetization components $\{M_x, M_y, M_z\}$, T_1 is the longitudinal relaxation time constant, and T_2 is the transverse relaxation

time constant. Denote the direction of the external superconducting Zeeman field, B_0 , as the z-direction. For the spin-echo pulse sequence, the sign of the water magnetizations along x- and z-directions was inverted by the 180° pulse at $TE/2$. For the CPMG pulse sequence, the sign of the water magnetizations along x- and z- directions was inverted by the 180° pulses at τ_{CP} , $3\tau_{CP}$, $5\tau_{CP}, \dots$ where $\tau_{CP} = 8$ ms. For the spin-locking pulse sequence, a constant locking field was applied along the y-direction.

4.8. Simulation Method 2 – Phase Accumulation Method

For the spin-echo and the CPMG pulse sequences, since the water magnetizations evolve entirely on the transverse plan, the phase accumulation method was used to significantly speed up the calculations [14, 26, 47], with additional modification to include background transverse relaxation. At each time step, Δt , every water magnetization experienced different induced magnetic field which depended on its position $\bar{r}$ and underwent a phase shift, $\Delta\phi(\bar{r}) = \Delta\omega(\bar{r}) * \Delta t$. The accumulated phase shift, Φ , was calculated by summing up individual phase shift produced at each random-walk step:

Equation 4.

$$\Phi = \sum_{n=1}^{\text{step}} \Delta\phi(\bar{r}_n)$$

The accumulated phase shift for the j^{th} water magnetization, Φ_j , gave the information on the rotation of the j^{th} water magnetization on the transverse plane. With additional phase factor, $e^{i\Phi}$ or $\cos(\Phi)$, the average water magnetization of the voxel, $S(t)$, was determined as:

Equation 5.

$$S(t) \approx \left| \frac{\sum_{j=\text{magnetization}} e^{i\Phi_j(t)}}{\sum_{j=\text{magnetization}} e^{i\Phi_0}} \right| = \left| \frac{\sum_{j=\text{magnetization}} S_j(t)}{\sum_{j=\text{magnetization}} S_0} \right| = |\langle s_j(t) \rangle|$$

where Φ_0 denotes the initial phase on the transverse plane after the first $\pi/2$ excitation pulse.

Here we assumed $\Phi_0 = 0$. The additional weighting factor of $\sin(\theta)$ over the vessel-cylinder angle θ and the sign flip of magnetization components by the 180° pulse were identical to the previous method.

4.9. Simulation Method 3 – ODE Solver

The evolution of the water magnetizations were obtained by numerically solving the Bloch equation with the Matlab ordinary differential equation solver, ode45. This method is accurate, robust, but very time-consuming.

5. Conclusions

In conclusion, spin systems of *in vivo* orthotopic GBM mouse models infected with U87MG cell line and orthotopic GBM rat models infected with C6 cell line were prepared by the spin-echo, the CPMG, and the spin-locking pulse sequences to high contrast states and then acquired by the fast spin-echo pulse sequence. For early-stage GBM mouse models (within 15 days after U87MG cells implantation), statistical results ($N = 18$) showed that, while the spin-echo T_2 -weighted MR imaging may not provide the required contrast to detect early GBM, the CPMG T_2 -weighted MR imaging with short inter-pulse spacing (*e.g.*, $\tau_{CP} = 1$ ms) and the spin-locking $T_{1\rho}$ -weighted MR imaging with high locking-field (*e.g.*, $B_1 = 2000$ Hz) slightly improve early GBM contrast but at the cost of significantly elevated specific absorption rate (SAR) and increased potential risk, if applied to human patients.

To facilitate the development of innovative MRI pulse sequences to enhance early GBM contrast with acceptable SAR, a simple theoretical model on early GBM was proposed based on the BOLD mechanism and the experimental observation that vessel density decreases together with the increase of vessel size during the early stage of the GBM growth. Using the proposed model, Monte Carlo spin dynamics simulations were carried out by solving the Bloch equations for the water magnetizations diffusing in the magnetic-field gradients induced by paramagnetic deoxyhemoglobin in the vessels under specific RF pulse sequences with various algorithms for computational efficiency and accuracy. The interplay among the spin dynamics, GBM contrast, RF pulse sequences used for imaging, vessel distributions, and GBM staging can be numerically evaluated. The simulated early GBM contrast and the relaxation time constants, T_2 and $T_{1\rho}$, from the spin-echo, the CPMG, and the spin-locking RF pulse sequences were compared with those

from the *in vivo* MR imaging of orthotopic early GBM mouse models to study the relaxation mechanisms. Our simulation results showed semi-quantitative and qualitative agreement with *in vivo* MR images during the GBM growth and thus this simple model provides a computationally efficient alternative for laboratory use of animals following the 3R (reduction, refinement and replacement) strategy in evaluating the performance of commonly use or newly developed MR methods for early GBM detection. Among various innovative MRI methods, we are particularly interested in and committed to the active-feedback methods based on non-linear spin dynamics [38-43], as have been demonstrated in subcutaneous colon cancer case [44]. Further applications of the active-feedback and fixed-point methods to GBM early detection are currently under intensive investigation in our lab. The proposed theoretical model shall be refined to incorporate more detailed information and complicated dynamics on early GBM such as the vessels becoming more disorganized and randomly oriented when GBM grows, changes in the diffusion coefficient of water magnetizations, the increase of vascularization in the periphery, and the MT effect.

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