PROBING THE EFFECT OF MICROSTRUCTURE ON WATER DIFFUSION IN ENGINEERED OR BIOLOGICAL MATERIALS WITH MRI

BY

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DISSertation
Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Mechanical Engineering in the Graduate College of the University of Illinois at Urbana-Champaign, 2013

Urbana, Illinois

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ABSTRACT

Water diffusion within skeletal muscles is a complex mass transport process due to the presence of barriers at multiple scales and the anisotropic structure of muscles. Magnetic Resonance Imaging (MRI) is an effective non-invasive technology for studying water transport in biological or engineered systems, and has been increasingly used to probe water diffusion in muscle. Because of the disparity between the length scales over which diffusion operates and MRI spatial resolution, the use of model systems is required in order to develop the methodology for deriving quantitative information from MRI data. Some engineered materials and systems share certain structural or functional characteristic with skeletal muscles and therefore can be used as model systems. For example, certain hydrogels that can change size shape or mechanical properties in response to solvent concentration have been used to fabricate artificial muscles. Bundles of aligned carbon nanotubes (CNTs) can be used to mimic the effect of the organization of myofibrils on water transport. Finally, the extraction of mass transfer properties from MRI data requires the integration of the underlying mass diffusion process with MRI imaging physics.

In this thesis, the diffusion-weighted MRI protocol is used to probe water diffusion inside a swelling Polyethylene Glycol Diacrylate (PEG-DA) hydrogel and in
the interstitial water-filled spaces in a bundle of aligned CNTs. Additionally, the diffusion-weighted MRI signal resulting from transversely anisotropic myofibers is simulated. The technical deliverables include: (1) A relationship between local water diffusivity and water concentration for PEG-DA hydrogel, which can be used to describe hydrogel actuator dynamics; (2) Water diffusivity parallel and perpendicular to the CNT orientation, which can be employed in the characterization of the intertube space; (3) Development of a two-compartment model for mass transfer in myofiber, which can account for anisotropic water diffusion in skeletal muscle.

In general, sub-voxel modeling and numerical simulation can facilitate the exploration of microscale structure using MRI when the structure is characterized by short range order. The reported experiments on these engineered materials not only contribute to the development of MRI methods for probing skeletal muscle, but also help advance their own intrinsic applications and understand the limitation of MRI for probing such materials.
To my family
ACKNOWLEDGMENTS

First and foremost, I would like to express my deepest appreciation to Prof. John Georgiadis, my academic advisor. John offered me a great opportunity to pursue my doctoral work, guided me to interdisciplinary areas, and provided me with an excellent atmosphere for doing research. I would also extend my gratitude to my advisory committee, Professor Narayana Aluru, Professor Brad Sutton and Professor Gaurav Bahl for their encouraging words, thoughtful criticism, and time they dedicated to review this thesis during their busy semester.

In addition, I have been very privileged to work with many great friends: Dimitrios Karampinos, Curtis John, Marios Georgiou, Lucija Rakocevic, Rafael Hernandez, Ouyang Cheng, Howon Lee, and Boris Odintsov. This study would have been much more difficult without their help and contribution. I also acknowledge support from the National Science Foundation, under grants CTS-0120978 and GBET-1236451, and the National Institutes of Health under grant HL090455.

Finally, I am greatly indebted to my family for their unconditional love, encouragement, and support during this long journey. They are always there to cheer me up and stood by me through good time and bad.
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CHAPTER 1

INTRODUCTION

1.1 Introduction

Mass diffusion in heterogeneous system with microstructure is complex transport phenomenon, because the process usually involves multiple spatial and temporal scales. In these systems, molecular diffusion is hindered or restricted by various diffusion barriers such as membranes and pre-space geometry and the observed apparent diffusion coefficients deviate from that of free diffusion. This deviation provides the opportunity to probe the structure at small length scale (Callaghan 1993). Magnetic Resonance Imaging (MRI) has proved to be a versatile investigation tool for heterogeneous diffusion, especially in biology tissues or microscale systems, because it is non-invasive and possess a wide variety of contrast mechanisms.

1.2 Background

1.2.1 Diffusion-weighted MRI

Water is the most commonly targeted molecule in proton MRI experiments in biological materials and it is the proton ($^1\text{H}$) spin of the water molecule can be
manipulated by the magnetic field of MRI scanners to produce the signal. Diffusion of water molecules causes a phase dispersion of the transverse magnetization of these spins, resulting in attenuation of the MRI signal. The degree of signal decay depends on tissue type, structure, physical and physiological state, as well as the water molecule microenvironment. By using specially designed protocols such as the Pulse Gradient Spin Echo (PGSE), MRI images with diffusion-weighted contrast that is sensitive to microscopic motion can be acquired. Since the resolution of diffusion-weighted MR images is usually not fine enough to distinguish microstructure directly, incorporation of prior knowledge about the underlying structure within each voxel explain the signal decay. The potential of diffusion-weighted MRI can be fully realized when applied to anisotropic or restricted diffusion system, although it has been initially used to quantify free diffusion.

1.2.2 Water diffusion in anisotropic and hierarchical structure – skeletal muscle

Tissues with anisotropic and hierarchical structure are very common in the human body, such as skeletal muscle, or the central nervous system. Specifically, human skeletal muscle has hierarchical organization in both the extra- and intra-myocellular domains, and this structure is intimately related to its primary function in terms of producing active stress. Figure 1.1 shows the structure of human skeletal muscle on different levels.
As shown at Figure 1.1, there are at least three levels of extra-myocellular organization: muscle fiber (which consists of parallel myofibrils, each contains thin/thick...
filament), muscle fascicle, and muscle organ. These structures are delineated by the sarcolemma, endomysium, perimysium, and epimysium which collectively comprise the extracellular matrix that ensures that the whole muscle contracts as a unit.

The connection between muscle function and local structure needs to be understood. Diffusion weighted MRI experiments (Cleveland et al. 1976), specifically employing Diffusion Tensor Imaging (DTI) (Basser et al. 1996, Damon et al. 2011) of skeletal muscle, have concluded water diffusion is highly anisotropic, and the apparent water diffusion coefficient parallel to the main muscle fiber direction is higher than in the transverse plane. Furthermore, the diffusion in the transverse plane is also anisotropic. However, the reason for this transverse diffusion anisotropy is still under debate, because the barriers of water diffusion inside and outside the myofiber are not clearly understood (Karampinos et al. 2009).

Each skeletal muscle fiber consists of a large number of parallel arrays of interleaved actin-myosin lattice (Huxley et al. 1954, Huxley et al. 1954). The arrays of the actin-myosin filaments, together with non-filament proteins, dissolve in cytoplasmic water and create diffusion barriers on various scales. Experiment results reveal that water diffusion parallel to the filament array is also 40% lower than bulk diffusion (Cleveland et al. 1976). The obstruction effect of filaments and non-filament proteins is not sufficient to account for the reduction in the spin-diffusion coefficient; hence another possible explanation is that large fraction of hydration water might form a substantial water
sheath around actin-myosin filaments and increase their size effectively (Cleveland et al. 1976).

Water diffusion also changes during skeletal muscle contraction (Grazi 2008). Spin-spin relaxation time T2 of skeletal muscle is known to increase after intensive muscle contraction, which is an indication of increased intracellular water content (Fisher et al. 1990, Nygren et al. 2002). Increased water content in exercising muscles affects both extracellular and intracellular volumes (Raja et al. 2006, Sejersted et al. 2000, Sjogaard et al. 1985), although the latter was presumed to be more important factor in T2 increase (Polak et al. 1988). This change, predominately increased water motion in the extracellular compartment, increased the mean Apparent Diffusion Capacity (ADC) of the muscle fiber, although the effects of cytoplasmic motions are yet not clear (Nygren et al. 2002). Hence, during muscle contraction, water content and muscle structure are mutually and dynamically related: water diffusion contributes to the alternation of muscle structure, which in turn affects the local water diffusivity.

1.2.3 Water transport in engineered system: deforming hydrogels and Carbon nanotube bundles

A strong correlation between water content, water diffusivity and medium microstructure can also be found in hydrophilic polymer networks that are classified as hydrogels. Certain hydrogels can swell or contract in response to a wide range of external
stimuli, and they resemble biological systems, such as muscle or tissues cells in the sense that they have similar structural integrity and provide material pathways that allow water and small molecules to diffuse through.

When a dry hydrogel comes in contact with water, the hydrophilic forces of the polymer network cause the expansion of the polymer chains to accommodate the water molecules. Such expansion of the network leads to increased water mobility, hence the gel will continue swelling until the polymer chains are fully expanded and saturated with water. Figure 1.2 shows a typical swelling process of hydrogels from dry state to fully swollen state.

![Figure 1.2. Polymer network swelling with water molecular diffusion (Lee 2011).](image)

Because of the unique properties and similarity to muscle, hydrogels have been used to create artificial muscle (Madden et al. 2004, Shahinpoor 1995, Shahinpoor 2003, Shahinpoor et al. 1998, Shahinpoor et al. 2000, 2004), which can mimic the function of skeletal muscle. Although, technically, the mechanism of muscle contraction is very different from simple polymer chain expansion or shrinkage like in hydrogels, the latter
can be used to study the dynamic effect of water content and local microstructure on water diffusivity in muscle. For hydrogels, the relationship between local water content and local water diffusivity is essentially necessary to understand or model the hydrogel swelling kinetics.

Another novel engineered system that has similarities with skeletal muscle is a carbon nanotubes bundle. Specifically, vertical aligned Carbon nanotube (VA-CNT) films exhibit certain features that mimic hierarchical structure inside the myofiber. Scanning electron microscope (SEM) images of VA-CNT show that nanotubes aggregate into crystalline ropes, and ropes group into high order structures, just like the myofibrils in myofibers. Additionally, when water molecules interact with the CNT walls, the first layer of water molecules next to the walls are organized in energetically favorable packing, according to molecular dynamics simulations (Marti et al. 2003). The hydration water sheath around filament protein of myofiber can be induced by similar inter-molecular forces. Water diffusion within CNT film can help probe inter-tube space and the film morphology, which is pivotal towards understanding the formation of CNT composites (Wang et al. 2007).

1.3 Diffusion-weighted MRI to characterize hydrogel and CNT

Since water is the ubiquitous solvent used in hydrogels, it is not surprising that proton NMR and MRI methods have been employed in this area. NMR experiments in
hydrogels (Baille et al. 2003, Kwak et al. 2003) involve the application of pulse field gradient to detect self-diffusion of small molecules and macromolecules in gels. These NMR experiments usually measure the average diffusion coefficient over the whole sample. The application of MRI enables the study of gel swelling kinetics, by acquiring series of proton density or $T_2$ maps to capture the water diffusion front with gels (Cranitch et al. 2007, Naji et al. 2008, Tritt-Goc et al. 2002). To probe the relationship between local water content and local diffusivity, one needs to acquire co-registered proton-density-weighted images and diffusion-weighted images (since the former provides information of local water concentration while the later provides local water diffusivity (Raguin et al. 2006). In MRI system, the spin echo sequence and PGSE sequence can be used to acquire these two images.

To probe the inter-tube space within CNT film, water can be used as a tracer to detect the tortuosity and pore structure of the film. Tortuosity is the ratio of the effective (apparent diffusion coefficient) in the medium over the bulk (free) fluid diffusion coefficient. The PGSE sequence has been proven to be effective in quantifying water diffusion within nanoporous zeolite microstructures (Banas et al. 2005). By choosing appropriate diffusion times, it has been shown that it is possible to distinguish four types of restricted spaces based on the following three characteristic lengths (Stallmach et al. 1999): root mean square displacement, spin dephasing displacement, and typical pore size. At long diffusion times, a clear differentiation between open and closed interstitial
space appears, and the tortuosity of the porous medium can be measured (Zielinski et al. 2003).

1.4 Thesis outline

This thesis focuses on the quantification of diffusion in heterogeneous media using diffusion-weighted MRI Pulse Gradient Spin Echo (PGSE). The feasibility of using PGSE to describe water transport in skeletal muscle-like engineered material or system, whose structure is either variable due to its interaction with water, or highly anisotropic and hierarchical, is explored. Finally sub-voxel modeling and simulation is used to explain the anisotropic diffusion of skeletal muscle on the plane normal to myofiber.

The remaining body consists of five chapters:

Chapter 2 contains necessary background in the implementation of PGSE. The Lattice Boltzmann (LB) method, which is the sub-voxel modeling scheme in this thesis is also briefly presented.

Chapter 3 describes the MRI experiments on Polyethylene Glycol Diacrylate (PEGDA) hydrogels, which focus on measuring and correlating the local water content and local water diffusivity.

Chapter 4 is dedicated to the MRI measurement of water diffusion within vertically aligned Carbon Nanotube film, which has an anisotropic structure.
Chapter 5 presents the sub-voxel modeling and simulations of NMR signal from transversely anisotropic myofibers and discuss the connection with a simplified multi-compartment model.

Finally, Chapter 6 summarizes the work presented in this dissertation and discusses opportunities for future studies.

### 1.5 References


Madden, J. D. W., et al. (2004). "Artificial muscle technology: Physical principles and


Tritt-Goc, J., et al. (2002). "Magnetic resonance imaging study of the swelling kinetics of

hydroxypropylmethylcellulose (hpmc) in water." Journal of Controlled Release 80(1-3): 79-86.


CHAPTER 2

PRINCIPLES OF DIFFUSION-WEIGHTED MRI
AND SUB-VOXEL MODELING OF DIFFUSION IN
HETEROGENEOUS MEDIUM

2.1 Introduction

The NMR signal, which underlines the physical principle of Magnetic Resonance Imaging (MRI), relies on the interaction between applied magnetic fields and specific nuclei with unpaired electrons, such as hydrogen $^1\text{H}$ (proton) or carbon $^{13}\text{C}$. Although the behavior of nuclear spin exposed to these fields can be understood in depth based on quantum mechanics, it can also be described using the classical vector model, which considers aggregates of nuclear spins, called isochromats. An isochromat is a collection of spins belonging to the same species and found in the same chemical environment, averaged over the atomic scale but small relative to the spatial scale of the applied magnetic fields. The following discussion focuses on non-interacting proton spins, since water is the most commonly targeted nucleus used in MRI experiment.

The hydrogen nucleus contains one positively charged proton, which is a spin-1/2 particle with an intrinsic magnetic moment corresponding to the quantum dipole. The
distribution of isochromats for these spins is described macroscopically by the spin density function $\rho$, and the state of isochromat at each spatial location is given by the magnetic moment $\vec{M}$, which is the ensemble average of all the quantum dipoles. The orientation of individual magnetic moment is randomly distributed owing to thermal fluctuations, unless it is exposed to a strong static external magnetic field $B_0$. The Varian machine used in the experiment described in this thesis imposes a field of $B_0=14.1$ Tesla, which is about 310,000 times stronger than that of the Earth's magnetic field. In the $B_0$ field, most of the spins will align with the $B_0$ direction and a bulk magnetic moment appears (thermal equilibrium magnetization). This magnetic moment vector precesses around the axis by $B_0$ (say z-axis) with the Larmor frequency:

$$\omega_0 = \gamma_0 B_0.$$  \hspace{1cm} (2.1)

where $\gamma_0$ is the gyromagnetic ration for the proton.

The same relationship between any externally applied magnetic field of sufficient duration and precession frequency applies. When a circularly polarized radiofrequency (RF) pulse of the same Lamor frequency is applied to the sample, the electromagnetic energy is absorbed into the spin system, and magnetic moment $\vec{M}$ tips toward the transverse plane (x-y). In MRI, this pulse is generated using an RF coil, which can also acts as a receiver coil to collect signal; this process is called excitation (or resonance) (Haacke et al. 1999). Figure 2.1 shows the procedure to produce a transvers component of magnetization $M_{xy}$. As a result of the excitation, $M_{xy}$ is precessing in the transverse plane.
The rotating $M_{xy}$ component generates a time-varying magnetic flux in the receiver and induces voltage, which constitutes the MR signal from the experiment.

Figure 2.1. (a) Random alignment of quantum dipoles. (b) Most spins align with main magnetic field $B_0$. (c) RF coil transmits energy to spins imparting a 90° flip angle. (d) Formation of transverse magnetization $M_{xy}$. (e) Precession of spin at Larmor frequency (Weishaupt et al. 2006).

In order to obtain an image in MRI, it is necessary to encode the spatial location of the spins with the signal. In certain MRI protocols, such encoding is achieved by
adding a magnetic field gradient across the sample. The signal from different locations has a correspondingly varying frequency component according to equation (2.2):

\[ \omega(r) = \gamma_0 (B_0 + \vec{g} \cdot \vec{r}), \]  

(2.2)

where \( \vec{g} \) is the magnetic gradient vector imposed by the user, and \( \vec{r} \) is the space vector. The image is essentially acquired in the spatial frequency (k) domain. When this signal is inverted using Fourier Transform, the image of the physical object is reconstructed. The application of spatially varying gradient also enables the excitation of specific layer or slice of the sample, using a RF pulse with corresponding finite bandwidth. Another useful concept is that of the Hahn spin echo, see figure 2.2. Right after the excitation pulse, all spins precess with the same transverse magnetization phase. Owing to the spin interaction with other spins, the initial phase coherence is lost. By arranging the RF pulse and gradients in an appropriate time sequence, the spins can be rephased and form a strong echo at a specific time \( T_E \). Specifically, a 180° refocusing pulse is applied at \( t=\tau \) to refocus all phase dispersion, hence the echo time \( T_E=2\tau \). The spin echo signal decays as \( \sim\exp(-T_E/T_2) \), and this can be used to estimate the spin-spin relaxation time \( T_2 \).

Besides spin-spin interaction, dephasing or MRI signal decay can be also caused by other factors, such as lattice relaxation, molecular diffusion, and fluid flow, etc. The image contrast caused by these factors includes rich information about the underlying phenomena, and render the MRI a versatile analytical tool. In order to extract this
information, the physics of the image formation under the specific contrast mechanism has to be taken into account. The following section will focus on three types of image contrast: T1-weighted, T2-weighted images, proton density-weighted images, and diffusion-weighted images.

![Diagram of Hahn spin echo sequence](image)

Figure 2.2. Typical Hahn spin echo sequence. Stage 1: initial state, only longitudinal component of bulk magnetization $M_z$. Stage 2: 90° RF pulse excitation, $M_z$ is flipped into transverse plane. Stage 3: spin dephasing due to T2 or T2*space decay. Stage 4: 180° refocusing pulse flips the spins, and spins reverse for $\frac{1}{2} T_E$. Stage 5: spin echo after full $T_E$ interval (Weishaupt et al. 2006).

### 2.2 Governing equations and images contrast

The precession of the magnetic spins is affected by their interaction with the local magnetic field caused by their surroundings and each other. Two time parameters are used to characterize such interactions, T1 and T2. The former is called ‘spin-lattice’
relaxation time, and it is used to describe in the process that the nuclei return to the
ground state by dissipating their excess energy to their surroundings. The latter is called
“spin-spin” relaxation and is used to describe nuclei exchanging energy with neighboring
nuclei. In practice, there is an additional dephasing of $M_{xy}$ introduced by external field
inhomogeneity, called $T_2^*$; but this dephasing can be recovered by applying a 180° RF
pulse.

The time-dependent behavior of total magnetization $\vec{M}$, under the influence of the
applied magnetic field ($\vec{B}_{\text{ext}} = (\vec{B}_0 + \vec{g}^* \vec{r})$) and with relaxation terms, can be described
by Bloch equation (Torrey 1956):

$$\frac{d\vec{M}}{dt} = \gamma \vec{M} \times \vec{B}_{\text{ext}} - \frac{1}{T_1} (M_0 - M_z) \vec{z} - \frac{1}{T_2} \vec{M}_\perp,$$

(2.3)

$$M_0 = \frac{\gamma^2 h^2 B_0 N_s \alpha}{16 \pi^2 k T_s}.$$

(2.4)

where $h$ is the Planck’s constant, $k$ is Boltzmann’s constant, $T_s$ is the absolute
temperature of the spin system, $N_s$ is the total number of spins, and $\alpha$ is a constant
determined by spin type. $M_0$ is called the thermal equilibrium value for $\vec{M}$ with the
presence of $\vec{B}_0$, $\vec{Z}$ and $\perp$ represent longitudinal direction and transverse plane
respectively and $\vec{B}_{\text{ext}}$ is the effective external magnetic field. The first term on the right
hand side of equation (2.3) corresponds to the spin precession due to external magnetic
field. The second and third terms correspond to T1 relaxation and T2 relaxation effects.

In the reference frame rotating around $\vec{Z}$ axis at the Larmor frequency (2.1), equation (2.3) can be written as:

$$\frac{\partial M_\perp}{\partial t} = -\frac{1}{T_1} (M_0 - M_z)$$

$$\frac{\partial M_\perp}{\partial t} = -i\gamma (\vec{r} \cdot \vec{g}^* (\vec{r}, t)) M_\perp - \frac{1}{T_2} M_\perp$$

where $\vec{g}^* (\vec{r}, t)$ is effective gradient, which incorporates RF pulse, and the term $-i\gamma (\vec{r} \cdot \vec{g}^* (\vec{r}, t))$ represents the phase change caused by the gradients. $M_\perp = M_x + iM_y$ is the complex magnetization in the transverse plane.

A typical MRI protocol involves repetition of time $T_R$ as shown at figure 2.2, in order to cover image k-space (Haacke et al. 1999) or to average the signal in order to improve the signal-to-noise ratio (SNR). When the RF excitation pulse is repeated, the transverse magnetization is continuously suppressed after every 90°RF pulse, and the magnetization components can be calculated by solving equation (2.5) with the following initial condition:

$$M_z (0^+) = 0$$

$$M_\perp (0^+) = M_\perp (0^-) = M_0$$

where $t=0$ is defined at the center of the first 90° pulse. For a spin echo signal resulting from a train of repeated RF pulses (neglecting the gradient dephasing term), the resulting
magnetization after n repetitions is (Stejskal et al. 1965):

\begin{align*}
M_z(nT_R) &= M_0(1 - e^{-T_R/T_1}) \\
M_\perp(2\tau + nT_R) &= M_0(1 - e^{-T_R/T_1})e^{-2\tau/T_2}
\end{align*}

Since \( T_E = 2\tau \) for spin-echo sequence, the magnetization magnitude at echo time can be obtained by replacing \( 2\tau \) with \( T_E \).

The expression of transverse magnetization in equation (2.7) indicates that the image intensity of spin echo sequence is influenced by \( T_1 \), \( T_2 \) and local proton density simultaneously. However, by choosing appropriate imaging parameters such as repetition time \( T_R \) and echo time \( T_E \), one can selectively emphasize one of them, while suppressing other contrasts. For example, imaging with short \( T_E \) and appropriate \( T_R \) leads to high \( T_1 \) weighting contrast, while appropriate \( T_E \) and long \( T_R \) generate \( T_2 \)-weighting images. A combination of short \( T_E \) and long \( T_R \) results in proton density-weighting images. Different contrast mechanisms give different information about the imaged object. \( T_1 \)-weighting and \( T_2 \) weighting can be used to image and identify microstructure or tissues and proton density-weighting images can be used to measure water content in the object.

## 2.3 Diffusion-weighted MRI and PGSE

As explained at the beginning of this chapter, linear magnetic field gradients can be used to encode spatial information into the MRI signal, which can be recovered to
form structural images during the reconstruction process. Gradients can be also used to sensitize image contrast caused by molecular motion during diffusion.

To take into account the diffusion effect on the signal, an additional term is added to equation (2.5), which now becomes the Bloch-Torrey equation (Torrey 1956):

\[
\frac{\partial M_\perp}{\partial t} = -i\gamma (\vec{r} \cdot \vec{g}^* (\vec{r},t)) M_\perp - \frac{1}{T_2} M_\perp + D \nabla^2 M_\perp. \tag{2.8}
\]

Here, D is the local diffusivity. Theoretically, D is a symmetric rank 2 tensor, but it is here simplified as a scalar. The solution of the above equation has the form:

\[
M_\perp (\vec{r}, t) = A(t) \exp[-i\gamma \vec{r} \cdot \vec{g}^* (\vec{r}, t) dt] \exp(-t / T_2), \tag{2.9}
\]

which accounts for both phase incoherence induced by field gradients and T2 relaxation. Substituting equation (2.9) into equation(2.8), an ordinary differential equation for the amplitude \(A(t)\) can be obtained as:

\[
\frac{dA(t)}{dt} = -\gamma^2 D \left( \int_0^t \vec{g}^* (\vec{r}, t') dt' \right)^2 A(t). \tag{2.10}
\]

The Solution of equation (2.10) with initial condition \(A(0) = M_0\) is:

\[
A(t) = M_0 \exp[-\gamma^2 D \left( \int_0^t \vec{g}^* (\vec{r}, t') dt' \right)^2 dt]. \tag{2.11}
\]

In MRI sequences, the magnetic gradients are selected so that the condition
\[ \int_0^{T_E} g^* (\vec{r}, t) dt = 0 \] is usually satisfied. Hence the transverse magnetization obtained at echo time \( T_E \) is:

\[
M_\perp (T_E) = M_0 \exp[-\gamma^2 D \int_0^{T_E} \left( \int_0^{T_E} \tilde{g}^* (\vec{r}, t') dt' \right)^2 dt] \exp(-T_E / T_2). \tag{2.12}
\]

If a diffusion decay factor is defined as:

\[
b = -\gamma^2 \int_0^{T_E} \left( \int_0^{T_E} \tilde{g}^* (\vec{r}, t') dt' \right)^2 dt, \tag{2.13}\]

then equation (2.12) becomes:

\[
M_\perp (T_E) = M_0 \exp(-T_E / T_2) \exp(-bD). \tag{2.14}
\]

Recall that equation (2.7) indicates that as 90°RF pulse is repeated, the transverse magnetization after each \( T_R \) is the results of tilting the longitudinal magnetization onto the transverse plane; hence it can be expressed by:

\[
M_\perp (T_E) = M_0 \left(1 - e^{-T_R / T_1}\right) e^{-T_E / T_2} e^{-bD}. \tag{2.15}
\]

It needs to be emphasized that, the analysis of diffusion weighting is simplified here. Unlike what is indicated in equation (2.13) and equation (2.15), \( D \) is a diffusion tensor and \( b \) is a matrix as for the mathematical formulation of Diffusion Tensor Imaging (DTI) (Basser et al. 1994).

The incorporation of diffusion-weighting in a spin echo sequence is usually
performed by adding a pair of identical gradient pulses (diffusion gradients), on each side of the 180° RF pulse, thus forming the Pulse-Gradient-Spin-Echo (PGSE) sequence. Figure 2.3 shows a typical Stejskel-Tanner PGSE imaging sequence and gradient echo sequence, which is used for numerical simulation in Chapter 5.

![Diagram](image.png)

Figure 2.3. (a) Stejeskal-Tanner PGSE sequence consisting of a spin echo sequence with addition of a pair of diffusion gradients on each side of the 180° RF pulse and imaging gradients. (b) Gradient echo sequence without imaging gradients used for numerical simulation.

Three parameters are used to describe the diffusion gradient pair: diffusion gradient amplitude $g$, gradient duration $\delta$, and spacing between the gradient pair $\Delta$. The diffusion decay factor (2.13) now becomes:
\[ b = \gamma^2 g^2 \delta^2 (\Delta - \delta/3) . \] (2.16)

Note that the effective diffusion time of this sequence is \((\Delta - \delta/3)\).

After obtaining a series of diffusion-weighting images with \(b=0\) and finite \(b\) values, the local diffusion coefficient can be extracted by fitting the formula:

\[ \frac{S(b)}{S_0} \sim \exp(-bD) , \] (2.17)

where \(S_0\) is signal acquired at \(b=0\) and \(S(b)\) is the signal corresponding to the specified \(b\) value. In the PGSE sequence, \(b\) values can be obtained by changing either the gradient amplitude \(g\) or diffusion times \((\delta\) and \(\Delta\)).

In complex heterogeneous samples, such as samples with permeable membranes, or restricted diffusion space, the Stejskal-Tanner equation (2.16) provides only an effective or Apparent Diffusion Coefficient (ADC) (Assaf et al. 2002). An alternative approach to analyze the diffusion-weighted MRI signal from such systems is the so-called q-space imaging (Callaghan et al. 1991, Cory et al. 1990), which can provide a rigorous method to extract structural information of the sample, or to quantify restricted diffusion (Li et al. 1997).

We conclude with a brief derivation of the previous results in terms of the so-called q-space formulation.

Based on narrow-pulse approximation \(\delta \ll \Delta\), molecular motion during the
gradient duration can be neglected (Callaghan 1993). A spin located at position $\mathbf{r}$, acquires a phase shift of $\gamma \delta \mathbf{g} \ast \mathbf{r}$ after the application of the first diffusion gradient.

Suppose that the spins move to a new location $\mathbf{r}'$ just before the second gradient is applied, due to molecular diffusion. A probability function $P(\mathbf{r}_1, 0 \mid \mathbf{r}_2, \Delta)$ is introduced to describe the probability of a spin moving from position $\mathbf{r}_1$ at time $t=0$ to $\mathbf{r}_2$ at $t=\Delta$. A new q-space variable, known as the reciprocal spatial vector, is defined as:

$$\mathbf{q} = \frac{\gamma}{2\pi} \int_0^t \mathbf{g}(t) dt = \frac{1}{2\pi} \gamma \mathbf{g} \delta.$$  \hspace{1cm} (2.18)

The normalized echo attenuation can be expressed as:

$$E(\mathbf{q}, \Delta) = \iint P(\mathbf{r}_1, 0 \mid \mathbf{r}_2, \Delta) e^{i2\pi \mathbf{q} \cdot (\mathbf{r}_2 - \mathbf{r}_1)} d\mathbf{r}_1 d\mathbf{r}_2.$$  \hspace{1cm} (2.19)

If one further assumes statistical homogeneity, then the displacement becomes independent of initial or final position so that $P(\mathbf{r}_1, 0 \mid \mathbf{r}_2, \Delta) = P(0, 0 \mid \mathbf{r}, \Delta)$. Equation (2.19) reduces to:

$$E(\mathbf{q}, \Delta) = \int P(0, 0 \mid \mathbf{r}, \Delta) e^{i2\pi \mathbf{q} \cdot \mathbf{r}} d^3 \mathbf{r}.$$  \hspace{1cm} (2.20)

Taking the inverse Fourier transform of equation (2.20) gives the ensemble average propagator (displacement probability profile):

$$P(0, 0 \mid \mathbf{r}, \Delta) = \mathcal{F}^{-1} (E(\mathbf{q}, \Delta)) = \int E(\mathbf{q}, \Delta) e^{-i2\pi \mathbf{q} \cdot \mathbf{r}} d^3 \mathbf{r}.$$  \hspace{1cm} (2.21)
By combing equations (2.16)(2.17)(2.18), the diffusion weighting signal decay for a rectangular Stejskal-Tanner gradient pair becomes:

\[
S(q) = S_0 \exp\left[ -4\pi^2 q^2 (\Delta - \delta/3) \right].
\]  

(2.22)

### 2.4 Practical considerations

All diffusion-weighting pulse sequences incorporate and use diffusion gradients to increase the sensitivity of phase distribution to water molecular motion. This motion causes a phase dispersion of the transverse magnetization, and results in the attenuation of the MRI signal. In terms of choosing the proper encoding method to maximize imaging quality, there are many factors that need to be considered when applying diffusion-weighted imaging, especially when imaging heterogeneous media (Bernstein et al. 2004, Le Bihan et al. 2006).

To achieve higher \( b \) factors, so as to increase the sensitivity of contrast to diffusion, a strong and stable scanner is required (Vavrek et al. 1995). When gradient amplifiers with large gradient intensities switch rapidly, the strong time-varying magnetic field generates eddy current in the MRI scanner. These eddy currents induce spurious magnetic field gradients, which degrades the magnetic field homogeneity and introduce geometric distortion into final images. Eddy current effects are compensated by
modifying gradient shape and timing (e.g. bipolar gradients), or post-processing the
results with correction to baseline images (Price 1998).

When MRI is applied to heterogeneous samples, internal gradient inhomogeneity
occurs because of the differences in magnetic susceptibility between porous matrix and
interstitial materials. These inhomogeneous gradients couple with the applied gradients
(imaging gradients and diffusion gradients) and introduce cross-terms artifacts in the
images. These susceptibility artifacts can be diagnosed by reversing the polarity of the
applied gradient (Latour et al. 1993, Moseley et al. 1991, Trudeau et al. 1995), and can be
minimized by using more sophisticated sequences (Corns et al. 1989, Price et al. 2001).

By design, diffusion-weighted MRI is more sensitive to motion artifacts,
compared with other MRI imaging technologies. Macroscopic motions (such as sample
movement during imaging) result in large amount of undesired phase shift in the image.
If all the spins within a given image voxel experience the same displacement or the
movement during imaging is known, the phase shift error can be corrected.

In order to conduct a successful MRI experiment, it is important to choose the
proper imaging parameters and achieve the highest possible signal-to-noise ratio (SNR).
However, diffusion-weighted MRI sequences tend to have low SNR because of the phase
incoherence caused by diffusion gradient (depending on the diffusion factor b), as well as
the long T_E required by the sequence. Long T_E is required by the constraints imposed by
the diffusion gradients. Although signal averaging can help increase the inherently low
SNR, a compromise needs to be made between long acquisition times and the image
qualities, on the basis that the minimum $T_R$ is also increased due to the introduction of diffusion gradients.

2.5 Sub-voxel modeling and Lattice Boltzmann Method

To quantify tissue fibers or microstructure, high resolution images are usually desired. On the other hand, diffusion weighted MRI experiments generally suffer from low spatial and temporal resolution limitations. This is not major problem, when diffusion weighting MRI is used to measure objects with coherent structures over multiple MR image voxels (like fibers in skeletal muscle or periodic CNT films). However, to understand the underlying mass transport within the voxels, it is necessary to establish sub-voxel models, which help to explore underlying microscopic structure and explain how it influences the MR signal. For example, to explain MR signal decay in heterogeneous system, multiple compartment models can be used to quantify underlying structure (Stallmach et al. 1999). Unfortunately, these models need to be connected with MRI physics. It is here that numerical simulation is required.

Various numerical simulation schemes can be applied to model water diffusion in heterogeneous system. The simulation of transporting complex porous media involves complex boundary conditions and multiple scales in both space and time. Since the numerical solution is usually computationally intensive, a numerical scheme which possesses the ability to easily incorporate multiple physics, flexibility in terms of...
boundary condition treatment, the potential to be implemented in a parallel computing environment should be preferred. The Lattice Boltzmann Method is used in the present work as a sub-voxel simulation scheme since it possesses such advantages.

LBM is a mesoscale numerical technology for solving partial differential equations (PDF), which has been used extensively in computational fluid dynamics, and mass transport problems. A comprehensive review of the LBM can be found in Chen (Chen et al. 1998). It has been shown that LBM is an accurate scheme for diffusion problems (Masaro et al. 1999, Naji et al. 2008, Raguin et al. 2006) using proper choice of relaxation time. The Lattice Boltzmann model employed here is the Bhatnagar-Gross-Krook model with single relaxation time, which simplifies the complex collision integral term in the Boltzmann equation by applying the linear relaxation approximation (Chen et al. 1998),

$$f_i(x + e_i \Delta t, t + \Delta t) - f_i(x, t) = \left( f^{eq}_i - f_i \right) \frac{\Delta t}{\tau}$$  \hspace{1cm} (2.23)

where \( f(x, v, t) \) is the single particle mass distribution function, \( x \) is the spatial position vector, \( v \) is the particle velocity vector and \( t \) is the time. \( f^{(eq)}(x, v, t) \) is the local equilibrium distribution function. A simple 2-dimension, 4-speed (D2Q4) model as shown in figure 2.4 is implemented in the simulation of diffusion.

The D2Q4 lattice model is composed by the following velocities:

$$e_i = (\cos \theta_i, \sin \theta_i)c \hspace{1cm} \theta_i = (i-1)\pi / 2, \hspace{0.1cm} i = 1 \sim 4$$  \hspace{1cm} (2.24)

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where \( c = \frac{\delta x}{\delta t} \) with \( \delta x \) is the unite mesh resolution, and \( \delta t \) is the time step, it expresses the distance over which information is communicated in a time step.

![Figure 2.4.Typical D2Q4 model for LBM simulation of diffusion problem.](image)

The equilibrium distribution equation can be written as:

\[
f^{(eq)}_i = \frac{1}{4} \ast M \quad i = 1 \sim 4
\]  \hspace{1cm} (2.25)

where \( M \) represents the macroscale variable. At the end of each LBM iteration, consisting of particle streaming and collision step described in equation(2.23), the macroscopic variables should be updated as:

\[
M = \sum_i f_i = \sum_i f^{(eq)}_i
\]  \hspace{1cm} (2.26)

In this model, the local relaxation parameter is related to the macroscale diffusion coefficient by:
\[ D = \frac{1}{2} \left( \tau - \frac{1}{2} \right) \frac{\delta x^2}{\delta t} \]  

(2.27)

The periodic boundary treatment in LBM is quite flexible and easy to implement (Chen et al. 1998). The diffusion problem in composite media has been simulated by adjusting the local relaxation parameter to accommodate a spatially varying diffusion coefficient (Naji et al. 2008).

### 2.6 References


CHAPTER 3

MRI MEASUREMENT OF NON-FICKIAN SOLVENT DIFFUSION COEFFICIENT IN SWELLING PEG-DA HYDROGELS

3.1 Introduction

Hydrogel are polymeric networks that can maintain a distinct three-dimensional structure without dissolving when they are swollen by water. The major constituent of hydrogels is water, which contained in a scaffold that consists of generally hydrophilic polymers cross-linked via chemical or physical bonds (Peppas et al. 2000). The ability of hydrogels to absorb water arises from hydrophilic functional groups attached to the polymer backbone while their resistance to dissolution arises from cross-links between network chains. Hydrogels are ubiquitous in different applications, such as tissue engineering (Ahearne et al. 2005, Elisseeff et al. 2001, Holland et al. 2005, Liu et al. 2009, Tan et al. 2009, Tibbitt et al. 2009, Yamaoka et al. 2006), implantable devices (Rahimi et al. 2011), contact lenses (Manetti et al. 2002), biosensors (Allcock et al. 2006, Brahimi et al. 2002, Koh et al. 2006, Massad-Ivanir et al. 2010), as a material for drug delivery (Hamidi et al. 2008, Luo et al. 2000, Wu et al. 2007), as well as in stimuli-
sensitive actuators (Bassik et al. 2010, Brock et al. 1994, Chan et al. 2012, Ismail et al. 2009, Kwon et al. 2010, Yew et al. 2007). Due to their capacity to entrap large amount of water, good biocompatibility and the ability to mimic live tissue environment, hydrogels are also receiving increasing attention as regenerative materials (Nimmo et al. 2011, Park et al. 2011, Perale et al. 2011).

Hydrogels can be classified based on the nature of their crosslinks, as chemical hydrogels when the crosslinks are covalent bonds, or physical hydrogels when the links are secondary weak bonds such as Van der Waals, electrostatic interactions, hydrogen bonds or molecular entanglements (Assunta et al. 2003). The crosslinks can be induced by radiation or chemical reaction. In the first case, the radiation reactions include electron beams, y-rays, X-rays, or UV light. In the second case of chemical crosslinking, the crosslink reaction can occur between small molecular weight crosslinking agents and a polymer chain. The crosslinking agent links two chains together through its di- or multifunctional groups, or a copolymerization-crosslinking reaction can occur between the monomers and a multifunctional monomer that is present in small quantities. It is very common to have a combination of the above mechanisms (Peppas et al. 2000).

On determining what materials are suitable for a specific application, the structure and properties of a hydrogel are extremely important; therefor the knowledge of the structure-property relationship is a prerequisite for tailoring hydrogel properties to the end goal. The specific polymerization methodology, curing temperature, cross-linker content, and the control of the gel swelling process affect the final gel properties such as
polymer fraction, polymer size and crossing density, the nanoporous structure inside the network. The method of hydrogel preparation can induce spatial inhomogeneity of pore structure and water content within the synthetic gel (Liu et al. 2000). The primary solvent transport mechanism within hydrogels is diffusion. The rate and distance a solvent molecule diffuses depend on both the material and intermolecular interactions with the tangled polymeric scaffold. Specifically for water as a solvent, the absorbed water molecules organize in various states, such as free, bound and interfacial water state (Lee et al. 1975), and this organization plays a significant role in determining the mass transport properties of hydrogels. The water uptake and swelling process in hydrogels is associated with dramatic volume change. The response history during swelling and shrinking of the gel is important in many applications, particularly in three dimensional (3D) active hydrogel actuators driven by swelling (Lee et al. 2009, 2010, Xia et al. 2008). In these devices, solvent is delivered to specific locations through embedded microfluidic channel or capillary networks built within the devices, for the purpose of local swelling control (Lee et al. 2010, Xia et al. 2009) and manipulation of 3D motion. The swelling kinetics of gels is controlled by the reformation of the polymeric network and diffusion of the solvent through the network. Hence a rigorous study for solvent migration into polymer is critical towards understanding and predicting micro actuation process and a model to describe the gel dynamics needs to couple the deformation of the network, constitutive relations, equilibrium, as well as kinetics of diffusion.
3.2 Types of diffusion in hydrogels and exponential diffusion model

When a hydrogel in its initial state comes in contact with solvent molecules (the swelling agent), the solvent (here water) diffuses through the hydrogel surface and penetrates into the polymeric network. The polymer chains change their conformation and expand, thus modifying the diffusion of other solvent molecules. This absorption process of polymer-solvent interaction does not conform to the classical theory of diffusion. Instead, the relation between the relative rate of penetrant diffusion and relaxation of the polymer chain can be used to categorize different types of absorption for the solvent. Alfrey et al. have proposed the following classification of the various hydrogel swelling processes (Alfrey et al. 1966, George et al. 2004):

1. Case I or Fickian diffusion: when temperature is kept above the glass transition temperature \(T_g\) of the polymer and the polymer is in rubbery state, polymer chains have a higher mobility, and allows easier penetration of the solvent molecule. Diffusion is significantly slower than the rate of relaxation of the polymer chains, and gel swelling is determined by the diffusion of the swelling agent. The diffusion distance is proportional to the square root of diffusion time.

2. Case II diffusion: when the temperature is below \(T_g\), i.e., the polymer is in glass state, the polymer chains do not have sufficient mobility to allow immediate penetration of the solvent into the polymer core. The rate of penetrant diffusion is higher
than the relaxation rate of polymer chains. The diffusion distance is directly proportional to time.

3. Case III or anomalous diffusion: Fickian diffusion and case II diffusion are the two extreme limiting types of the transport process. Anomalous diffusion lies in between, when both rates are comparable. The exponent of the time dependence of the diffusion distance lies between 0.5 and 1. Anomalous diffusion is also regarded as a subcategory of Case II diffusion in most literature discussing solvent diffusion in hydrogels.

Since most of hydrogel diffusion experiments are conducted in ambient temperature and typical $T_g$ for hydrogels are $80$–$90^\circ$C (Seidel et al. 2000), there is ample evidence on case II diffusion, and numerous mathematical models have been proposed describing the kinetics of hydrogel swelling (Argon et al. 1999, El Afif et al. 2002). Based on the physical meaning of completion between penetrant diffusion and polymer chain relaxation, Thomas and Windle (Thomas et al. 1982) proposed that viscosity and diffusivity of solvent depend on solvent uptake. Wu and Peppas (Wu et al. 1993) improved this model by modeling the gel as viscoelastic materials via a Maxwell model. Using these models, an exponential diffusion law can be derived, relating the diffusion coefficient to the volume fraction of the solvent. The following derives the mathematical model used in type III diffusion.

The solvent diffusion flux $j$ is driven by the gradient of the chemical potential (Feynman et al. 1963), i.e.
where D is the solvent diffusivity (unit length$^2$/time), \( k \) is the Boltzmann constant, \( T \) is the temperature and \( \phi(x,t) \) is the volume fraction of the solvent (volume of solvent per unit volume \( V \)). The chemical potential \( \mu \) (per solvent molecule) in the gel is given by:

\[
\mu = kT \left[ \ln \phi + (1 - \phi) + \chi (1 - \phi)^2 \right] + P v,
\]

(3.2)

The first term corresponds to the contribution of the mixing between the polymeric network and solvents (Flory 1953), where \( \chi \) is the Flory interaction parameter, and the second term is the external work done by the osmotic pressure \( P \) on a solvent molecule with volume \( v \). An equivalent form was also adopted by Hong et al (Hong et al. 2008).

Then the solvent flux is explicitly expressed as:

\[
j = -D \frac{v \phi}{kT(1 - \phi)(1 - 2 \chi \phi)} \nabla \cdot \boldsymbol{\sigma}^{\text{def}} + \nabla \phi,
\]

(3.3)

when a mechanical equilibrium condition is established inside the gel:

\[
\nabla \cdot \sigma = 0, \text{ and } \nabla \cdot \sigma^{\text{def}} = \nabla P \text{ in } V,
\]

(3.4)

\( \sigma \) is the gel stress, and \( \sigma^{\text{def}} \) is the gel stress due to the deformation of the polymeric network, and
\[
D_{12} = \frac{\phi D \partial \mu}{kT \partial \phi} = D(1-\phi)(1-2\chi \phi)
\]  

(3.5)

is the mutual diffusivity (Wu et al. 1993) and the equilibrium equation (3.4) has been applied. Equation (3.3) indicates that flux is driven by both the gradient of gel stress resulted from the deformation of the polymeric network and the volume fraction of solvent. The dependence of mutual diffusivity \( D_{12} \) on solvent concentration can be expressed in the following exponential form (Thomas et al. 1982, Wu et al. 1993):

\[
D_{12} = D_0 \exp (a_d \phi),
\]

(3.6)

where \( D_0 \) is the initial diffusion coefficient (diffusivity of solvent into the dry polymer) and \( a_d (>0) \) is a phenomenological parameter that describes the solvent concentration dependence. The empirical expression (3.6) can be characterized from experiments and phenomenological parameters can be measured.

Various techniques have been used in the study of diffusion in polymer solutions and gels, such as gravimetry (Hu et al. 1996), membrane permeation (Smith et al. 1988), and fluorescence (Wisnudel et al. 1996) and dynamic light scattering technologies (Van Asten et al. 1996). The rapid development of Magnetic Resonance Imaging (MRI) technology provides an additional non-invasive tool to investigate and measure diffusion properties. MRI has been used to characterize water diffusion within different hydrogels, either to capture the diffusion front dynamically (Naji et al. 2008, Tritt-Goc et al. 2002)
or to measure diffusivity and water content (George et al. 2004, Manetti et al. 2002, Raguin et al. 2006). In this study, MRI measurement is used to establish the relationship between local water content and local water diffusivity of Polyethylene Glycol Diacrylate (PEG-DA) hydrogels, which have been used in fabricating 3D micro actuators and structures (Lee et al. 2009, 2010, Xia et al. 2008). Two different MRI protocols were used to measure local water content and diffusion coefficient in specially prepared swollen hydrogel plugs, as described below. The obtained parameters are fed into the proposed empirical expression (3.6) for further modeling use.

3.3 MRI experiment on proton density and diffusivity of PEG-DA gel

3.3.1 PEGDA hydrogel sample preparation

All the hydrogel samples are prepared by Dr. Howon Lee, who has been a postdoctoral scholar in Professor Nicholas Fang’s research group at Massachusetts Institute of Technology (MIT). Porous PEG-DA hydrogels were synthesized by mixing PEG-DA pre-polymer (MW575, Sigma Aldrich) with PEG (MW200, Sigma Aldrich) in a weight ratio of 1:3 followed by addition of 0.5% wt. of photo-initiator (phenylbis (2,4,6-trimethylbenzoyl) phosphine oxide, Sigma Aldrich) for photo-polymerization under UV
illumination (λ=365nm). Not being polymerized, PEG contributes to reducing crosslinking density by occupying intermolecular space between PEG-DA during photopolymerization, resulting in low modulus and large swelling ratio.

The sample preparation procedure is illustrated in Figure 3.1.

Figure 3.1. PEG-DA gel preparation procedure.

First, cylindrical master was fabricated by projection micro-stereolithography (Sun et al. 2005), using hard polymeric material, hexanediol diacrylate (HDDA) (step 1). Thin metal layer was coated on the master (step 2), followed by saline treatment for easy demolding process (step 3). Then poly(dimethylsiloxane) (PDMS) was poured and cured for 4 hours at 80°C to make transparent complementary mold (steps 4-6). The PDMS mold was filled with pre-polymer solution and illuminated for 10 s in UV oven, followed by another 10 s exposure after flipping over for uniform crosslinking. A gel rod was then retrieved out of the PDMS mold (step 7) and put in acetone bath for rinse for 3 hours, followed by 1 hour dry in a vacuum desiccator. Finally, the dry hydrogel rod was inserted
into MRI glass tube (Norell 509-UP, 5mm diameter), and swelled in DI water, and the ends of the tube were sealed with plastic cap.

By varying the design of the mold, i.e. the diameter of the dry hydrogel rods after fabrication, different water concentration profile within the hydrogels can be achieved. Two types of designs were used during the experiment, uniformly cylindrical master, and tapered cylindrical master as shown in photo of step 1 in Figure 3.1. The former first is used to generate uniform swelling profile along the axial direction, while the second is used to generate a continuous water concentration profile within the same sample, continuously varying gel from dry state to swollen state. Water diffusion inside the gel is isotropic, and as the gel swells bigger, the glass tube confines the swelling in the radial direction. Hence, the tapered mold design provides varying water concentration, compared with the uniformly cylindrical mold. In order to guarantee that all data are acquired using the same MRI experiment parameters, as well as for saving data acquisition time and sample preparation cost, the tapered mold design was used to acquire a wide range of solvent concentration data from a single sample. To further confine the gel from swelling in the axial direction and obtain data from lower water concentration values, glass rods were inserted into to glass tubes to limit the swelling. Figure 3.2 shows one gel sample without axial confinement, and while a second sample with glass rods on both sides of the gel plug. The gel plugs are about 2cm long after swelling, while the glass tube holding the sample is around 6cm long, so as to reduce the
magnetic susceptibility artifact introduced by the plastic top during MRI scanning.

Figure 3.2. PEG-DA hydrogel sample plugs in tube. Axial confinement is employed in the lower sample.

3.3.2 MRI protocol to measure local water volume fraction and water diffusivity

The MRI protocols to extract local water content and local diffusion coefficient in the PEG-DA hydrogels are similar to the one used to probe diffusion in 2-Hydroxyethyl Methacrylate (HEMA) hydrogels (Raguin et al. 2006). The experiments were conducted at the Biomedical Imaging Centre at the University of Illinois at Urbana-Champaign (UIUC), using the wide-bore 14.1 Tesla (600mHz) vertical imaging system (Oxford Instruments, Abingdon, UK, shown at figure 3.3), equipped with a gradient set (Resonance Research, Billerica, MA) capable of gradient strength up to 100G/cm in 0.1 ms. All experiments were performed on a Unity console (Varian, Palo Alto, CA).
A standard spin density spin-echo sequence (“sems” in VNMR 6.1C software) was used to extract water content, and a standard pulse-field gradient spin-echo sequence (“semsdw” in VNMR 6.1C software) was used to measure the local water diffusivity. In the diffusion-weighted image, the signal intensity in each voxel can be expressed as equation (3.7):

\[ I \propto w \exp\left(-\frac{T_E}{T_2}\right) \ast [1 - \exp\left(-\frac{T_R}{T_1}\right)] \ast \exp(-bD), \quad (3.7) \]

where \( w \) is the spin density, \( T_R \) is the repetition time, \( T_E \) is the echo time, \( T_1 \) and \( T_2 \) are the spin-lattice and spin-spin relaxation times respectively, \( D \) is the local water diffusion coefficient (isotropic), and \( b \) is the diffusion sensitivity factor (Callaghan 1993).

Hydrogel samples were imaged together with a glass tube of a mixture of deionized (DI) water and Deuterium Oxide (D2O) mixture (at 20%, 40%, 60%, 80%, 100%
volume fraction of DI) as reference. The T1 relaxation times for the hydrogels samples and DI water are less than 1.5s and close to 2s respectively, as measured using the standard NMR saturation recovery sequence “s2pul” in VNMR 6.1C software. The DI/D$_2$O mixtures were doped with copper sulphate to bring their T1 down to about 1s. The signal intensity in the hydrogels was compared with those from the reference tubes to extract the water content of the hydrogel. To save time for repeated scanner tuning and shimming, as well as to make sure all images are acquired in the same magnetic files, several hydrogel samples were imaged together during a single experiment. The hydrogel tubes are positioned around the DI/D$_2$O reference tube, and then all samples are wrapped up and placed in a holding plastic tube, which was inserted in the RF coil. Figure 3.4 shows a typical configuration of the samples inside the holding tube.

![Figure 3.4. Hydrogel samples and reference tube inside a plastic test tube.](image)

A rubber ring is used to adjust the position of the sample inside the RF coil.

Several capillary tubes filled with copper sulphate solution were placed inside the holding
tube as markers to help identify samples. After an image on the transverse plane was acquired first to locate samples, appropriately chosen image slices through the gels and the reference tube were defined in order to conduct water content and diffusivity measurements. Figure 3.5 shows a typical transverse plane image and a targeted slice from the experiment.

The PED-DA hydrogels used in this study were prepared differently from the HEMA hydrogels in the prior study (Raguin et al. 2006). HEMA and DI water was mixed at specific nominal volume fraction, and then this pre-polymer was allowed to polymerize under controlled temperature. The PEG-DA hydrogels used in our study were polymerized in dry state by photochemistry, and then hydrated with DI water, so the actual amount of water absorbed during the swelling procedure is unknown.

Figure 3.5. (Left) Transverse image of all samples, in the center was a 40% H2O/D2O mixture, and the lower right sample didn’t show up because of low SNR for this sample. (Right) Acquired slice for water content and water diffusivity.
3.4 Results

Due to the lack of prior work related to MRI measurements of PEG-DA hydrogels, two additional experiments were carried out to verify the applicability of spin-echo sequence to measure water content and water diffusivity in PEG-DA gels. The objective of first one was to measure water content in PEG-DA hydrogel sample, prepared in a way similar to those HEMA samples (Raguin et al. 2006), while the second focused on calibration of the water diffusivity measurement. The remaining of this section summarises the MRI results obtained for establishment of the relationship between water content and water diffusion in the swollen hydrogels.

3.4.1 Measuring PEG-DA hydrogel with known water content

To prepare PEG-DA hydrogel with known water content, water was mixed with pre-polymer solution before crosslinking, then liquid-state pre-polymer of known volume water concentration was added in glass tube, cured under UV and the tube was sealed with end caps. Figure 3.6 is a photo of the sample with known water concentration. In Figure 3.6, the water content varies from 5% in sample 1, to 80% in sample 4. As it can be inferred from texture, the mixture of water and the pre-polymer solution become less homogeneous when water concentration rises above 50%, due to the fact that the solubility of water in the pre-polymer solution is limited. In sample 3, for example, the texture is reminiscent of a droplet emulsion.
These gels samples were imaged together with 40% DI/ D2O mixture reference tube using the spin-echo sequence, and the result was used to extract the water concentration based on equation(3.7). Instead of using Carr-Purcell-Meiboom-Gill sequence (CPMG, codified as “cpmgt2” in the VNMR 6.1C software) to measure the T2 of the whole gel, T2 maps of the gels were obtained by acquiring several spin-echo images with different echo time (T_E = 40ms, 80ms, 160ms, 320ms, 640ms). The T2 of the reference mixture was found to be is 385.47ms±5.54ms. Then the T2 values for these gels were calculated by averaging the T2 on voxels within selected regions of interest (ROI). Table 3.1 summarizes the T2 values for the four gel samples.
Table 3.1. Measured relaxation time $T_2$ for PEG-DA gels with specified water volume fraction.

<table>
<thead>
<tr>
<th>Water content in gels</th>
<th>5%</th>
<th>10%</th>
<th>50%</th>
<th>80%</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_2$ (ms)</td>
<td>43.96</td>
<td>55.1</td>
<td>135.21</td>
<td>218.18</td>
</tr>
<tr>
<td>Error (ms)</td>
<td>2.14</td>
<td>1.94</td>
<td>14.45</td>
<td>7.57</td>
</tr>
</tbody>
</table>

The parameters for the spin-echo imaging experiment were as below: $T_R$=6s, $T_E$=11ms, 126×256 data points in k space acquired over 3cm×6cm of field of view (FOV) and a single slice with thickness of 1mm, resulting in a voxel size of 234μm ×234μm × 1mm. Experiments were conducted using the Varian Doty coil. Long $T_R$ and short $T_E$ were utilized to acquire proton density-weighted images (Callaghan 1993). Figure 3.7 shows the acquired image of sample 2 and sample 3, and the computed proton density map of the samples.

The emulsion feature in sample 3 can also be observed in Figure 3.7. Proton density maps are used to calculate the water concentration within the gels, and the results are summarized in Figure 3.8. As shown at Figure 3.8, the obtained water volume fractions extracted from the spin-density-weighted images agreed with the known values, within an error of less than 1% for samples 1 and 2. Sample 3 and sample 4 exhibits large error due to solubility problem discussed above.
To further examine whether this discrepancy is caused by the acquisition procedure or the samples themselves, as well as to calibrate the mixture reference, the same procedure were carried out for all the DI/D$_2$O mixture (with water volume fraction of 20%, 40%, 60%, 80%), and a tube of pure DI water. The results are shown in Figure 3.9. Figure 3.9 indicate that the measurement agrees with the known mixture composition with an error of less than 1.5%.
Figure 3.8. Spin-density measurements of PDG-DA gels with known water content. The values indicate measured value ± error percentage.

Figure 3.9. Spin-density measurements of DI/D2O mixtures of known composition. The values indicate measured value ± error percentage.
3.4.2 PGSE Measurement of Hydrogel Diffusivity

The diffusivity of PEG-DA hydrogel is measured with a diffusion-weighted spin echo sequence. The accuracy of the diffusivity measurement was estimated by measuring the water diffusion coefficient within a pure DI sample and within a fully-swollen sample. The experiment was conducted with the same Varian standard coil and “semsdw” sequence. All the parameters are below: $T_R=5s$, $T_E=40ms$, FOV=$5mm \times 5mm$, matrix size=64x64, slice thickness=1mm. Since diffusion within the hydrogels is isotropic, the diffusion gradient was only applied along the phase encoding direction, by setting dro=0, dpe=1, and dsl=0. $\Delta=30ms$, $\delta=2ms$. The diffusion gradient $g_{diff}$ was arrayed into 11 steps, ranging from -60G/cm to 60G/cm, which resulted in a range of the diffusion sensitivity factor $b$ from 0 to about 3(ms/$\mu m^2$). The signal was averaged on ROI placed in the center of the tube. Subsequently, the water diffusivity was extracted by fitting the signal decay to a monoexponential function. The results for DI water and PEG-DA hydrogels are plotted in Figure 3.10 and Figure 3.11, respectively.

Only the five low b-value pairs of data on (Left) plot of Figure 3.10 were used in the (Right) plot to perform curve fitting. The highest b-value data was not used because of low signal-to-noise-ratio (SNR). The curving fitting result obtained in Figure 10 gives the following values for water diffusion coefficient: $D = (2.0538 \pm 0.00238) \times 10^{-9} m^2 /s$, which agrees with prior experimental results (Mills 1973, Weingaertner 1984) within a 2.6% error.
Figure 3.10. MRI measurement of water diffusion coefficient within DI sample. (Left) signal acquired from experiment shows exponential decay. (Right) curve fitting of the experimental results.

Figure 3.11 show the diffusion-weighted MRI signal decay and curve fitting corresponding to a highly swollen PEG-DA gel. The curve fitting results give $D = (1.25561 \pm 0.00369) \times 10^{-9}$ m$^2$/s for water diffusion coefficient in the hydrogel.

Figure 3.11. MRI measurement of water diffusion coefficient within PEG-DA hydrogel. (Left) signal exponential decay. (Right) curve fitting of the result, the slope give the water diffusivity.
To the best of our knowledge, there have been no other reports of diffusivity measurement in PEG-DA hydrogels, comparing the present results with a theoretical study based on the use of molecular dynamics to investigate effect of cross-linking on the diffusion of water in PEG-DA hydrogels (Wu et al. 2009), shows good agreement within error of 10%.

### 3.4.3 Correlation Between Water Content and Water Diffusivity

After verifying that both the spin-density sequence and diffusion-weighted spin-echo sequence can accurately measure the water concentration and water diffusivity in PEG-DA proxy gels, respectively, the same procedure was applied to the swelling PDG-DA hydrogels, which were photopolymerized in dry state and then hydrated with water.

After the proper slice identified on the basis of transverse plane image of the sample, two sequences, ‘sems’ and ‘semsdw’, were executed consecutively on the same field of view (FOV), so that proton density-weighted images are co-registered with diffusion-weighted images. To quantify spin density, sufficiently long $T_R$ and shot $T_E$ should be applied to eliminate $T_1$ and $T_2$-weighting effects. The $T_2$ map generated indicated that the shortest $T_2$ among all samples was about 83ms. Standard inversion recovery measurements show that the longest $T_1$ for swollen gels is about 1.76s in uniformly swollen gels prepared using cylinder mold. The $T_2$ map was taken into account
when calculating the water concentration, even though the T2 weighting effects were negligible with a short $T_E$ as implied by equation (3.7).

The following parameters were used to acquired proton density weighted images: $T_R = 8s$, $T_E = 17ms$, $3cm \times 6cm$ FOV, $128 \times 256$ data points in k-space, single slice of 1mm thickness. The same parameters were used in the diffusion-weighted images, with the exception of $T_R = 6s$, and the following additional parameters for diffusion gradient were used: dro=0, dpe=1, dsl=0. $\Delta = 30ms$, $\delta = 2ms$, and the diffusion gradient "gdiff" was arrayed into 7 steps ranging from $-60G/cm$ to $60G/cm$. Data were acquired from 2 sets of prepared PEG-DA gels and are summarized in Figure 3.12.

![Figure 3.12. Cumulative plot water diffusivity measurement for all swollen hydrogels.](image)
The abscissa of the plot in Figure 3.12 represents water volume fraction within the hydrogels, and the ordinate gives the measurement water diffusion coefficient. The notation of ‘Gradient sample set 1’ (small black square) and ‘Uniform sample set 1’ (blue dots with error bars) corresponds to normally swollen gels, ‘Gradient sample set 2’ (small red triangle) and ‘Uniform sample set 2’ (green dots with error bars) were obtained from swollen gels with glass rods confining axial swelling. The notation ‘Gradient’ and ‘Uniform’ refer to gels synthetized using taped cylindrical mold and uniformly cylindrical mold respectively. All the hydrogel samples in set 2 had shorter length after swelling because of the axial confinement with glass rods; the lengths ranged from 1.0cm~1.2cm, compared with 2cm of normal hydrogels without axial confinement. This indicates that water diffused within smaller volume and was distributed more uniformly than those gels allowed to swell in the axial direction. This explained why results obtained from sample set 2 had apparently lower error than those from sample set 1.

As seen in Figure 3.12, the set 2 gradient sample only spans a relatively narrow range of water concentration, about 43% ~63%, and this was caused by the swelling process continuing during sample shipping from Boston to Urbana (1-2 days). A wider water concentration range was initially designed by fabricating sharply taped mold but the water concentration decrease with time.

Figure 3.13 shows a gradient PEG-DA hydrogel sample (with glass rods confinement) swelling from the dry state to the hydrated state within 3 hours. For PEG-
DA gels with this cross-linking density, the higher water concentration, the higher the optical opacity of the gel. A sharp transition region from opaque to transparent can be seen in the middle photo. After 3 hours of diffusion and swelling, this sharp front disappears.

Figure 3.13. Sequence of photographs demonstrating the stages that an originally dry tapered hydrogel sample (Left) undergoes as it swells. Note that the water distinction becomes progressively uniform (Right).

To use the result in model analysis, parameters in equation (3.6), parameter $D_0$ and $a_d$ need to be obtained from curve fitting. Figures 3.14 and figure 3.15 show the curve fitting results for the gradient and uniform samples, respectively. The first gives $D_0 = 0.1011 \pm 0.0023$, and $a_d = 2.8197 \pm 0.0278$; while the second results in $D_0 = 0.0512 \pm 0.0144$, and $a_d = 3.4327 \pm 0.3259$. The large error with the second fit is due to the relative sparsity of data points. The data will be used as input for building a dynamic model of PEG-DA hydrogel swelling in the future.
Figure 3.14. Curve fitting result for gradient hydrogel samples.

Figure 3.15. Curve fitting result for water diffusivity of uniform samples.
3.5 Discussion

Based on the assumption that water and hydrogel matrix volumes are conserved during the swelling process, i.e. that the volume increase of hydrogel from initial dry state to swollen state equals exactly to the volume of the water that entered, the volume fraction of water inside the hydrogel can be estimated from the simple formula:

$$ \phi = \frac{V_{\text{swollen}} - V_{\text{dry}}}{V_{\text{swollen}}} \cdot (3.8) $$

Photos of the hydrogel in dry state and swollen state were taken and used to estimate the volume change by measuring the change gel cylinder length and diameter. Then gel samples are assumed to be approximately perfect cylinders and formula (3.8) is used. Compared with the estimation of water volume fraction based on hydrogel dimension change, the MRI measurements of water content are consistently higher by 10%~35%.

Although a 10%~20% discrepancy between the MRI measurement result and the nominal water content was also observed in HEMA gel experiment (Raguin et al. 2006) using the same instrument, this was justified by the fact that the actual amount of water absorbed was less than the expected nominal value, and that excess water was observed in the sample vials outside the gels. Our prior measurement of PEG-DA gels prepared by pre-polymer solution mixing with water (section 3.4.1) also exhibits error less than 10%.

There are several possible factors that can explain the above discrepancy of the swollen hydrogels. First, the gels are assumed as uniform, which is not true for all gels.
Since the gels were not allowed to swell freely (confined radically by tube walls, and confined axially by glass rods), and the gels swelled were under stress, it is very hard to predict the uniformity of water content and to select the best ROI to eliminate measurement bias. In our experiments, only the core of the hydrogel images was used and this inevitably brings in non-uniformity bias errors. As seen in Figure 3.12, this error can be up to 10%~15% for swollen gels. Second, the estimation of water content based on sample column dimension change is not accurate enough. The assumption that the shape is a perfect cylinder tends to underestimate the total volume, because the interfaces are not truly flat as shown in Figure 3.16. This contributes about 5%~8% of the error. Third, the gels actually continue swelling after the samples are sealed inside the tubes. By comparing MRI images with those photos taken after swelling, it was found that the gels will be 0.5mm~1.5mm longer; this also contributed another 5%-10% error of the dimension estimation (normal gel length is close to 2cm), especially for the high water volume fraction case. Fourth there are errors in measurement results from using simplified formulas for the imaging parameters (Mattiello et al. 1994), but this is negligible.

Figure 3.16. Typical gel-water interfaces are not flat.
### 3.6 Conclusion

The volume fraction of water inside swollen PEG-DA hydrogels, and the corresponding water diffusivity were measured using spin-density images and diffusion-weighted sequences in MRI experiment. Different types of hydrogel samples with water concentration or uniform distribution, different swelling configuration (axially confined or not) were imaged to extract a correlation between the local water content and local water diffusivity. The data were curve-fitted and the results provided input parameters for an exponential diffusion model. Hydrogels provide a good example where the structure (polymer network) and the water diffusion mutually are coupled, and this might provide insight into MRI techniques for describing diffusion in muscle.

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CHAPTER 4

PROBING WATER DIFFUSION IN THICK CARBON NANOTUBE FILM

4.1 Introduction

The first identification of what we call carbon nanotubes (CNT) today was made by Sumio Iijima at the NEC Research Laboratory, with the aid of high resolution transmission electron microscopy (Iijima 1991). He observed a new type of finite carbon structure consisting of needle-like tubes, the so-called carbon multi-walled nanotubes (MWCNTs), while studying the soot made from byproducts obtained during the synthesis of fullerenes by the electric arc discharge method. Two years later, single wall carbon nanotubes (SWCNTs) were independently synthesized at IBM Almaden Research Center (Ajayan et al. 1993) and at NEC in Japan (Iijima et al. 1993), by adding metal particles to the carbon electrodes (cobalt was used at IBM and iron at NEC). Since then, the richness and diversity of the properties of CNT (chemical, electronic, thermal, and chemical) have provided the impetus for the scientific community to focus on CNT and on its potential applications.
CNTs are hollow cylinders rolled up from seamless graphite sheets, with diameter in the nanometer range. Figure 4.1 shows a typical schematic of SWCNT constructed from a grapheme sheet. The diameters of SWCNTs can be as small as 0.6~1nm and the length can be as long as millimeters. MWCNTs have similar lengths to SWCNTs, but much larger diameters, with inner diameter around 5nm, and outer diameter around 100nm corresponding to about 30 coaxial tubes (Reich et al. 2004).

Because of the symmetry and unique structure, CNTs have exceptional properties in different aspects. They are strongest and stiffest material per unit mass yet discovered in terms of tensile strength and elastic modulus (Peng et al. 2008), and when transformed into super-hard phase nanotubes, they have even higher bulk modulus than single diamond crystal (Popov et al. 2002). The inner nanotube core of MWCNTs nested within
outer nanotubes may slide almost without friction (Zettl 2006). Regarded as 1D electrical conductor, SWCNTs have excellent conductance. CNTs are reported to be very good thermal conductors (ballistic conduction) along their axis direction, but good insulator along the radial directions (Pop et al. 2006, Sinha et al. 2005). Based on these superior properties, an intensive and multidisciplinary research effort has been carried out during the past two decades to explore potential novel CNT applications. They have been used in synthesizing atomic force microscope probes (Hafner et al. 2001), bone cell proliferation and bone formation scaffolding in tissue engineering (Shi et al. 2007, Sitharaman et al. 2008), nanotube fabrics and sheets (Dalton et al. 2003, Zhang et al. 2005), bio-mimetic hierarchical composite materials (Koziol et al. 2007, Miaudet et al. 2005, Naraghi et al. 2010), nanotube-based transistors (Collins et al. 2001) and nanotube integrated memory circuits (Tseng et al. 2004), and solar cell panel (Guldi et al. 2005) and hydrogen storage media (Dillon et al. 1997).

4.1.1 Vertically Aligned Carbon Nanotube Film

By organizing individual nanotubes into a well aligned fiber bundles (Cui et al. 2000), their individual anisotropic properties can be further. Hence, in many applications such as nanotube based electronics and nanocomposites, nanotubes bundle aligned along a single direction are preferred. Figure 4.2 shows a typical well-aligned carbon nanotubes configuration.
Figure 4.2. An as-grown, dense, multi-walled CNT array produced with a Fe-catalyzed chemical vapor deposition process (Hinds et al. 2004).

The properties of the individual nanotubes, the building blocks for the fiber, and hierarchical organization of these bundles are crucial in determining the final properties of the whole CNT system.

Specifically, the rapid development of highly efficient catalytic processed has advanced the research in vertically aligned growth methods. Vertically aligned carbon nanotubes (VA-CNTs) has been employed in micro-electro-mechanical systems (Fang et al. 2005) and molecular separation membranes (Holt et al. 2006). Unusually high water flux, over three orders of magnitude higher than that predicted from continuum hydrodynamic models, through micro-fabricated membranes comprised of aligned double wall carbon nanotubes (DWCNTs) has been observed(Holt et al. 2006, Hummer et al. 2001). Such high flux was explained by the fact that CNTs’ hydrophobic walls are atomically smooth and allow considerable slip of water molecular through the pores, as well as the coupling of water molecules’ rotation-translation movement inside the
nanotubes (Joseph et al. 2008). In fact, nanoporous membranes based on CNTs have been shown promising in improving salt rejection and increasing product flux in water purification, by matching or exceeding the performance of commercially available nanofiltration membranes (Shannon et al. 2008). In nanocomposites applications, it was also shown that composites with aligned nanotubes exhibit higher mechanical strength compared to nanocomposites created using randomly oriented CNTs (Garcia et al. 2007).

4.1.2 Polymerization of as-produced CNT membrane

During the fabrication of nanotube-based membranes or nanocomposites, a polymer is injected and fills the intertube space, in order to form robust support substrate. Different kinds of polymer and polymerization methods have been tried for CNTs layer. Garcia et al. (Garcia et al. 2007) created CNT-epoxy nanocomposites by wetting CNT pillars (10nm in diameter, 40-70μm in length) with SU-8 epoxy (Microchem 2000.1 and 2025) using a submersion method. Hinds et al. (Hinds et al. 2004) spin-coated 50% weight-percent solution of polystyrene (PS) on the surface of 5-10 μm thick CNT film, and the film was impregnated with PS due to the high wettability of PS within CNT. High viscous drag within the CNT sustained the intertube polymer, while excessive polymer on top of the composite structure was removed during the spin-coating process. Holt et al. (Holt et al. 2004) used low pressure chemical vapor deposition (CVD) furnace to deposit low-stress silicon nitride onto a 5-10 μm thick MWCNT forest to form a
During this polymerization process, the transport properties of polymer inside CNT forest, the interaction of polymer molecules with CNTs, and the CNT layer morphology will all play important role in determining the finale quality of nanoporous membrane or nanocomposite structure. For example, during dispersion and embedding of bulk SWCNTs or MWCNTs in polymeric matrix, aggregates of CNTs tend to form that are poorly adhered to the matrix and concentrate stress (Thostenson et al. 2001), while polymer submersion (Garcia et al. 2007) or capillary-driven wetting (Garcia et al. 2007) of VA-CNTs preserve the initial alignment of the CNTs. The interaction between polymer chains and CNTs is another factor influencing the polymerization process. When the CNT diameter is very large, its wetting properties, which determines the interaction with polymers and other liquids, approach to those of graphite (Dujardin et al. 1994). But the diameter of CNTs and the intertube spaces usually fall in the nanometer scale (tens of nm or less), which is of the same order of magnitude with the radius of gyration (Rg) of most polymers (3-30nm), that quantifies the span of polymer chain in the solution and many related polymer properties(Winey et al. 2007). On this scale, polymer chains proximal to CNTs will be more perturbed by the interfacial forces from the CNT walls than from interactions with polymer in bulk. The perturbation is caused by variations in the degree of curvature, chain mobility, and crystallinity. This effect will become more significant when the CNT bundle become denser and the intertube space shrinks, as the
thickness of polymer interfacial layer surrounding individual CNTs is independent on the CNT diameter (Wardle et al. 2008). When the total volume of CNT arrays shrinks, the volume fraction of such interfacial region increases, and hence the collective polymer perturbation effect becomes increasingly important.

For the long CNTs, the filling process is poorly controlled due to the lack of understanding of the interactions between the external surface of the CNTs and the polymer chains. The hierarchical organization of the CNT needs to be quantified and studied in a way without interrupting the original structure, i.e. non-invasively. This problem is even more difficult for long aligned CNTs, since there tend to be more defects as individual CNTs grow longer and more voids could be formed during the polymerization process. As a result, the emphasis is placed on the probing of the transport properties of the intertube fluid in conjunction with the CNT packing morphology. Although long molecular polymers are typically used to impregnate into the CNTs layers, it is methodologically prudent to start with a simpler fluid, such as water.

4.1.3 Water Diffusion within CNT membrane

Since it has been shown that water can wet hydrophobic CNTs (Dujardin et al. 1998), and water motion is influenced by the interaction with CNT structure, the idea is to explore the CNT morphology indirectly by observing the water motion changes. Molecular
dynamics simulations (MD) have been carried out to study the water molecular interaction with single CNT (Walther et al. 2001) or bundle of CNTs (Marti et al. 2003), under the scenario that water molecules surround infinitely long CNTs. Their results show the diffusion transport properties are similar to the case when water is facing a graphene sheet. The water molecules are slightly inclined to the CNT walls, with the first layer of water molecule next to the wall packed in an energetically favorable pattern. Martin et al. (Marti et al. 2003) further predict that water molecules interacting with the exterior surface of CNT bundle exhibit a diffusion coefficient equal or higher than the water bulk diffusion coefficient, depending on the quantity of water molecule surrounding the CNTs. Although there have been measurements of water transport within CNT (Wang et al. 2008), no report of measuring water diffusivity within the intertube space exist, to the best of our knowledge. This chapter explores the use of Magnetic Resonance Imaging to probe intertube morphology and water-CNT interaction.

The choice of water as interstitial fluid naturally calls for the use of proton Nuclear Magnetic Resonance (NMR) or Magnetic Resonance Imaging (MRI). Most the NMR studies focused on characterizing water molecules transport within CNTs (Chen et al. 2008, Mao 2007, Mao et al. 2006, Sekhaneh et al. 2006, Wang et al. 2008). A noteworthy result from these measurements is that water confined within nanotubes has significant chemical shift, while the intertube space water has no chemical shift compared to bulk water, as Chen et al. (Chen et al. 2008) revealed in the NMR study of confined water within
SWCNTs. By measuring diffusion of the molecules occupying the inner space of porous structures, MRI has also proved to be efficient tool to probe morphology and structure of periodic microporous materials (Tomadakis 2007, Zielinski et al. 2003) and extracellular space structure (Nicholson et al. 1998). However, to our knowledge, there has been no application of diffusion-weighted MRI (DW-MRI) for water transport in CNT. In this chapter, DW-MRI is applied to measure water diffusivity within a long VA-CNTs and to connect with the CNT nanostructure by exploring the hierarchal organization of the nanotube bundles.

4.2 CNT synthesis and characterization

Synthesizing highly organized arrays of CNTs using bulk growth methods is the key to reduce the production cost and to promote wider applications. Many years of research endeavors have led to three main methods for CNT production, including arc discharge, laser ablation, and chemical vapor deposition (CVD). Among them, CVD has become the most important commercial method, because it can be easily scaled up to industrial production levels, and both synthesis of MWCNT and SWCNT using CVD has been well developed. CVD also demonstrates better control over the morphology and structure of the produced nanotubes (Joselevich et al. 2008). During the CNT production process of CVD, gaseous or volatile carbon compound is decomposed, the resulted vapor is
deposited on metallic nanoparticles which serve as nucleation sites on a substrate, and the
CNT growth is initiated. Most commonly used metal catalysts include Fe, Fe/Mo or Ni
with Al or Al₂O₃ support. By modifying various parameters of the CVD process, such as
catalyst composition, thickness of catalyst layer, carbon feedstock, pretreatment time,
growth temperature and growth time, flow of the vapor, CNTs with desired characteristics
(such as length, diameter, and nanotube density) can be produced.

In order to grow long VA-CNTs film used in the DW-MRI experiment reported
here, the CVD process developed by Dr. Olgica Bakajin’s group at the Lawrence
 Livermore National Lab (LLNL) was employed, and a number of samples were prepared
by Ms. Lucija Rakocevic from the Lab of Quantitative Visualization and Energetics at
 UIUC (Rakocevic 2009). In the LLNL protocol, metallic catalyst is composited of a 10nm
thick layer of Al sputtered on silicon or quartz wafer, and 0.5nm layer of Fe and 0.3nm
layer of Mo deposited by e-beam evaporation. The wafers are cut into 1cmx1cm pieces and
heated in a furnace. By adding Al layer, the average CNT diameter could be better
controlled (Kayastha et al. 2007). When the Al layer melted around 660°C, Fe/Mo
nanoparticles are submerged within the resulted liquid. The liquid meniscus serves two
purposes: to prevent the nanoparticles from aggregating and to limit the area of
nanoparticles available for CNT growth. In this fashion, both the CNT density and
nanotube diameter could be manipulated.
Figure 4.3 shows a schematic of this fabrication procedure. A 12 minutes pre-treatment with H\textsubscript{2} was carried out to remove available O\textsubscript{2}, which reduces the oxidization of metallic nanoparticles, and improves their wetting by the liquid Al layer (Seidel et al. 2004).

After catalyst nanoparticles are formed, the growth of carbon nanotubes is initiated by releasing ethylene into the furnace, which serves as the carbon source gas. Exposed to high temperature at 750\textdegree C, ethylene is chemically decomposed and starts forming a carbon monolayer on the catalytic nanoparticles. Since the formation of CNT is more energetically favorable than a capsule of the same size, addition of carbon atoms will lead to the continuous growth of nanotube (Hafner et al. 1998). To extend the lifetime and activity of catalytic particles, and sustain the “root-growth” of nanotubes, water is added in as weak oxidizer to remove amorphous carbon coating on the particles. All the long VA-CNTs that were used in this study were fabricated following this protocol with the following
parameters: CNT growth for 1hr with 80sccm (standard cubic centimeter per minute) of ethylene flux, 15scmm of H₂ flux, 400sccm of Ar flux and 4sccm of Ar~H₂O mixture flux. To terminate the growth process, all gases were turned off except for Ar, which is used to cool down and decrease the residual concentration of ethylene. CNT growth stopped as the temperature dropped and most of the residual ethylene turned into amorphous carbon, resulting in impurity on the CNT film (Yamaguchi et al. 2008). Figure 4.4 presents a time record of the CVD process according to the above protocol.

Figure 4.4. CVD procedure for growth of 1mm long VA-CNTs (Rakocevic 2009).
The remaining of this section contains materials from the MS thesis of Ms. Rakocevic and is included here only as a reference for the MRI measurement reported in subsequent sections.

The lengths of the produced CNT film fell in the range of 300µm to 1.2mm depending on several factors, such as positioning inside the furnace tube, coverage of catalyst tri-layers on the wafer surface, etc. After finishing the CVD process, the produced CNT layer is removed from the substrate using a standard razor blade. Some of the catalyst nanoparticles will remain on the CNT film during this cutting procedure. The purity of the CNT films was then investigated using Inductively Coupled Plasma (ICP), and their structure and organization were also investigated using Raman spectroscopy, Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM). A 16ppm Fe within the obtained CNTs was found from ICP measurement, which indicated that most of the Fe catalyst nanoparticles were still attached on the CNT film after they were removed from the substrate. Hence the CNT film is processed through a chemical cleaning procedure. Micro-Raman spectroscopy with laser excitation wavelength at 633nm was used to characterize the nanostructure of the CNT films in this experiment. Three typical Raman spectrums of our samples are presented in figure 4.5.
The Raman spectrum shows three main peaks: radial breathing mode (RBM) which appears only with presence of SWCNTs, disorder induced (D-band) which is caused by grapheme structure disorder on the CNT walls and amorphous carbon materials in the sample, and tangential mode (G-band) is induced by graphite structure of CNTs (Jorio et al. 2003, Zdrojek et al. 2004). The spectrum shows that the obtained CNT film is associated with semiconducting nanotubes and their structure and organization is not uniform along the film.
the film depth. Direct imaging of the film using SEM and TEM further confirmed the non-uniform characteristics.

SEM and TEM images of the CNT sample were taken separately using a Hitachi S-4800 High Resolution SEM scanner and a JEOL 2100 Cryo TEM scanner at the Material Research Laboratory of UIUC. Some of the TEM images are presented in figure 4.6, showing the observed SWCNT with diameter around 2~10nm. Figure 4.7 shows the observed residual catalyst nanoparticles close to the root of the film, and figures 4.8 presents SEM images of observed organized structure of nanotube in different scale.

Figure 4.6. TEM images showing SWCNT structure with diameter around 5nm in the film.
Figure 4.7. Observed residual nanoparticles around the root of the CNT membrane.

Figure 4.8. Different scale CNT structures observed under SEM scanner. The resolution of the images increased from (a) at 1mm to (d) at 500nm.
Figure 4.7 indicates that the chemical cleaning could not remove all purities, especially for the region close to the growth substrate. By comparing SEM images on the top, middle and bottom of the film, it was also found that the nanotube alignment is worst at the bottom area, and the nanotubes tend to be curvier and entangled (Rakocevic 2009).

4.3 Architecture and simplified diffusion model of CNT as a porous medium

Due to the strong intertube Van der Waals attraction, as-produced SWCNTs tend to align parallel to each other and pack into crystalline ropes (Bandyopadhyaya et al. 2002, Fischer et al. 1997, Gogotsi et al. 2006, Thess et al. 1996) and the ropes are then organized into bundles. This organization was also observed in the CNT film used in this experiment, as shown at Figure 4.8 (c). Referring to the hierarchal structure of the human skeletal muscle (Saladin 2010), there are certain structural analogies between CNTs and myofibrils, CNT rope and muscle fiber, and CNT rope bundles and fascicles as shown in Figure 1.1. There are of course strong functional differences that muscle fibers are covered by sarcolemma, which is an active and selective mass transport barrier, and myofibrils and fascicles are constructed by complex networks of proteins and other macromolecules.
Given the complex porous structure of CNT membrane, Monte Carlo simulation (Latour et al. 1994) for intertube diffusion, coupled with Molecular Dynamics simulation for the interactions between water and CNT outer walls could be used to estimate the effective water diffusivity over different scales. Precisely because of the presence of disparate length scales, this simulation would be prohibitively complex and time consuming. The objective here is to provide a parameterization of the MRI results in terms of sample structure parameters, instead of simulating the diffusion precisely, so simple porous media models (Whitcomb et al. 2002) are employed.

Inside the film, CNTs form two-dimensional triangular lattice through Van der Waals bonding, with lattice constant at 1.7nm (Thess et al. 1996). Assuming a nanotube diameter of 5nm, the estimated CNT volume fraction for a single CNT rope is 50.5%. As the CNT wall is impermeable to water and the CNT interior is sealed to water during the fabrication, the nanotubes can be consider as voids in a continuum water matrix. If the interfacial interaction between water molecules and CNTs is neglected, the effective water diffusion coefficient for a single rope can be calculated: $D_{\text{rope}} = 1.16 \times 10^{-9} \text{ m}^2/\text{s}$, based on the volume fraction of the impermeable fibers arranged in Hexagonal array (Whitcomb et al. 2002). Let us assume that each CNT rope has diameter of 50nm, and these ropes aggregate into bundles of ropes on a bigger scale, with diameter of 50nm, with spacing between ropes of 50nm, and on triangular lattice configuration. Hence the volume fraction of CNT ropes inclusion embedded within the continuum water matrix is 22.67%. From
Maxwell equation (Alvarez et al. 1996), which gives a good approximation when the volume fraction is low, the estimated effective water diffusivity on the bundle of ropes scale is \( D_{\text{rope}} = 1.787 \times 10^{-9} \text{ m}^2/\text{s} \). Finally, on the highest level structure, an aggregate of bundles composed of 200nm big ‘rope of ropes’, spaced 200nm away from each other and arranged in triangle layout. The volume fraction of this inclusion is 22.67%, with a resulting diffusivity of \( D_{\text{brope}} = 1.95 \times 10^{-9} \text{ m}^2/\text{s} \).

In order to interpret MRI diffusion experiment in heterogeneous systems, different multi-compartment models have been proposed (Kärger et al. 1988, Pfeuffer et al. 1998). As shown at SEM images, if CNT ropes are regarded as a compartment with restricted diffusion in connected porous space and the space between bundles of ropes as another compartment with free (unrestricted) diffusion, the CNT film can be model as a simple two-compartment model (Kärger et al. 1988), and the water exchange between these two compartments is instantaneous (without barriers). Figure 4.9 shows a schematic of such simplified model.

![Figure 4.9. Two-compartment model of CNT films based on the SEM images.](image)
Using the simplest Kärger model (Kärger et al. 1988), the total NMR signal in a two-compartment system, as a superposition of the weighted signals from the individual components, can be expressed as:

\[
S = S_{\text{free}} + S_{\text{restricted}} = v_{\text{free}} \exp(-bD_{\text{free}}) + v_{\text{restricted}} \exp(-bD_{\text{restricted}}),
\]

\[
v_{\text{free}} + v_{\text{restricted}} = 1,
\]

where \( v \) is the volume fraction of the two compartments separately, and \( D \) is the local water diffusion coefficient. Restricted diffusion in connected porous space is a manifestation of the tortuosity of the ropes of CNTs in a bundle of ropes. And by changing the diffusion time for the diffusion weighted imaging time, the measurement of \( D_{\text{restricted}} \) will also reflect CNT structure parameters, because the water molecule will act as markers to probe all the available interstitial space.

### 4.4 MRI diffusion experiment in CNT films

The synthesized CNT films grown in LLNL were measured using the 14.1T Inova Varian MRI scanner in Beckman Imaging Center at the University of Illinois at Urbana-Champaign. The as-grown film was submerged in 5% ethanol solution for 24 hours to hydrate the CNT surface. After hydration, a tiny piece of the film was cut from the film and rinsed with deionized (DI) water for another 12 hours to make sure water diffuse into
inter-tube space. The sample was subsequently placed inside quartz tube (OD 1mm, ID 0.7mm, and length 4cm). DI water was filled into the glass tube, and the tube was sealed with soft wax.

One of the difficulties with MRI measurements in the CNT films is the low signal-to-noise ratio (SNR), which is a consequence of the small size of the sample and difficulties with the hydration of the inter-tube space. In order to improve the SNR, a 2mm ID microcoil (designed and built by Dr. Boris Odinotsov, Beckman Institute at the University of Illinois at Urbana-Champaign) was used in the reported experiments. This microcoil has a better filling factor (Hoult et al. 1976), which is important for small samples. The CNT sample is placed with the CNT axis in the x-y plane, which is perpendicular to the main magnetic field direction $B_0$. Figure 4.10 shows a schematic of how the target slice is selected, and a typical image of the slice when $b=0$. As seen at figure 4.10, the CNT plug (gray band) is surrounded by DI water (bright band).

Figure 4.10. (Left) schematic of imaging slice and diffusion gradient directions. (Right) typical image of the slice when $b=0$. 

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A standard diffusion-weighted spin echo sequence (labeled ‘semsdw’ in the VNMR 6.1C software) was used to measure the diffusion coefficient. The following imaging parameters were used unless specified otherwise: $T_R = 3\text{s}$, $T_E = 40\text{ms}$, field of view of $8\text{mm} \times 8\text{mm}$, and matrix size of $64\times64$. The slice thickness is $1\text{mm}$, which covers the whole CNT plug. The diffusion gradient was applied along the phase encoding (dpe) and slice selective direction (dsl) to probe diffusion parallel and perpendicular to the CNT axis respectively. 33 different gradients (arrayed from $-80\text{ G/cm}$ to $+80\text{ G/cm}$ with step increment of $5\text{G/cm}$) were applied to obtain signals corresponding to different $b$ values. Diffusion gradient duration $\delta$ was set at $2\text{ms}$ in order to fulfill the short pulse approximation requirements so that spin motion during $\delta$ can be neglected.

In order to investigate the dependency of observed diffusivity $D$ on diffusion time $\Delta$, experiments with $\Delta$ set at $7\text{ms}$, $15\text{ms}$, and $30\text{ms}$ were performed. Since the effective diffusion time of PGSE sequence is $(\Delta - \delta/3)$, the corresponding characteristic root mean square (rms) displacements of water molecules can be calculated as follows:

$$l_{\text{Diff}} = \sqrt{2D(\Delta - \delta/3)} .$$

Since the free diffusion coefficient of water under the experiment temperature ($20^\circ\text{C}$) is $2.038 \times 10^{-9} \text{m}^2/\text{s}$ (Mills 1973), the corresponding rms displacements are $5.08\mu\text{m}$, $7.64\mu\text{m}$, and $10.93\mu\text{m}$. The dephasing length $l_{\text{phase}}$, over which diffusion of the molecules will cause significant disturbance ($2\pi$ radians) of the originally introduced spatial distribution
of phases, is calculated as follows (Stallmach et al. 1999):

\[ l_{\text{phase}} = (2\gamma \delta \gamma)^{-1}. \]

(4.4)

Another similar definition to calculate this dephasing length is proposed to be (Hürlimann et al. 1998):

\[ l_g = (D_0 / \gamma g)^{1/3}. \]

(4.5)

In the reported experiments, the shortest dephasing length is 7.3μm (by the former definition) or 3.8 μm (by the later definition). The mean pore radius <R> in this experiment would have possible choices of 1.7nm, 50nm, or 200nm; the inter-tube or inter-rope spaces. Obviously, \(<R> \ll l_{\text{Diff}}\) and \(<R> \ll l_{\text{phase}}, l_g\); hence the actual rms displacement is the pore space is smaller than in the bulk fluids, and it is too short to incur significant disturbance to the phase distribution. This implies that the diffusivity measurements are very sensitive to the interstitial space (Stallmach et al. 1999).

Bulk water diffusion can also be measured in the imaged slice, and can be used as a reference value to check the measurement accuracy.

4.5 Results and statistical analysis

The apparent diffusion coefficient can be estimated by the signal decay as D~
ln(S/S_0)/b, according to equation (2.17). Figure 4.11-4.13 shows typical nonlinear signal decay obtained from the diffusion-weighted MRI experiment with diffusion time 7ms, 15ms, and 30ms.

Figure 4.11. Results for non-shrunk sample with Δ=7ms.

Figure 4.12. Results for non-shrunk sample with Δ=15ms.
As seen on these figures, the ln(S/S₀) vs. b curve deviates from linearity as the diffusion time and the diffusion weighting factor b increased, which is a sign for restricted diffusion (Le Bihan 1995), and indicates that the diffusion coefficient is sensitive to the structure of the CNT film. However, the non-linear behavior could also be caused by a putative internal gradient induced by the spatial variation in sample magnetic susceptibility. This susceptibility gradient may not be negligible at high magnetic field, because it couples with the main magnetic field and induces a cross-term in the obtained signal (Endre et al. 1984), which affects the accuracy of the diffusion coefficient measurements. Hence its significance needs to be analyzed before proceeding further. The strength of susceptibility depends on the difference in magnetic property of water and CNT film. CNTs at room temperature are diamagnetic with susceptibility of
about \(-9.60 \times 10^{-6}\) emu/g \(-10 \times 10^{-6}\) emu/g (Ramirez et al. 1994), compared to water with susceptibility of \(-9.05 \times 10^{-6}\) emu/g. According to Hurlimann et al. (Hurlimann 1998), the effective dephasing length defined by the magnetic susceptibility can be calculated by

\[
\tilde{\ell} = \left( \frac{D_0}{\gamma \Delta \chi B_0} \right)^{1/2},
\]

(4.6)

which determines whether the pore size is small enough that the spins average out the internal field inhomogeneity. This length for the present experiments is estimated at 2-2.5 \(\mu\)m, which is larger than the characteristic length of the pore structures. Hence internal gradients owing to susceptibility differences are not the dominant reason for the non-linear curves in Figure 4.12 and 4.13.

It is also noticed that the diffusion results for positive and negative diffusion gradient do not overlap. This discrepancy is most obvious for the diffusion within CNTs when the gradients are applied along the nanotube direction. Since susceptibility effect is also larger along the nanotube direction, this disagreement between the results could be attributed to the cross-term effect from the internal gradient or the cross-term from the imaging sequence itself (Zaric et al. 2004). To correct this effect, a simple algorithm was adopted (Güllmar et al. 2005), where the image acquired with positive gradient results and the images acquired with negative gradient are combined into single image by using:
Figure 4.1 and 4.15 compares the original data and the cross term correction. The red lines are results obtained by simply averaging the ± gradient branches. As shown at the figures, the cross term treatment using equation (4.7) reduces the statistical error significantly. This effect is most obvious when the diffusion gradient is applied along readout direction of the system, but these data are not discussed.

![Diagram of diffusion coefficient](image)

Figure 4.14. Statistical analysis on bulk water diffusion data with cross term correction. Diffusion gradient is applied along phase encoding direction.
Since the CNT film is generally a heterogeneous nanoporous structure, the acquired data needs to be further analyzed statistically. For this set of experiments, voxel size is 125 µm×125 µm ×1mm, which is much larger than the size of ‘bundles of ropes’, the largest length scale in the CNT film. Since the CNT films were prepared with a process which ensures uniform growth over the whole substrate surface, we assumed that there is statistical homogeneity over voxels. Furthermore, the results are averaged over two neighboring voxels, covering an area of 125×250 µm². Because the sample size is small (usually 30-35 voxels are available), the t-distribution test is used for the statistical analysis, with the confidence level set at 99.5%. The image signal S is sampled as independent variable and the dependent variable is the diffusivity D, calculated from equation (2.17). Figures 4.16-4.18 present the statistical analysis results for diffusivity.
As seen from these figures, the bulk water diffusivity normal to nanotube directions is consistently lower than that along the tube direction (about 8%), even when the region of interest (ROI) for water is not close to the CNT plug. This discrepancy exists within all acquired data, hence there is a systematic error. Probable causes are gradient asymmetry or the generation of eddy currents owing to high switching rate of diffusion gradients. It is easy to justify that water diffusivity within CNT membrane is anisotropic, with the water diffusivity parallel to CNT axis being higher than that normal to it. Furthermore, this trend should become more pronounced when diffusion time increases, or the diffusion factor b increases. The measured difference between the diffusivity values along the two directions is close to 5%. Adjusting for the systematic error mentioned above, this difference could increase to 13%.

Figure 4.16. Diffusion in non-shrunk sample at Δ=7ms.
To confirm that the diffusivity measurement is really sensitive to structure on the transverse plane, a modified CNT sample was also used. The sample originated from the
same CNT film but it was subsequently manually condensed and shrunk to increase the nanotube packing density. The cross sectional area is about 20% less than that of non-shrunk samples. Figures 4.19-4.21 show the water diffusivity obtained from the shrunk sample.

Figure 4.19. Diffusion in shrunk sample at $\Delta=7\text{ms}$.  

Figure 4.20. Diffusion in shrunk sample at $\Delta=15\text{ms}$.  

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Figure 4.21. Diffusion in shrunk sample at $\Delta=30$ ms.

Because of additional problems with hydrating the shrunk sample, the SNR for this shrunk sample is lower than the non-shrunk, and this is reflected in the higher error seen in the figures. Nevertheless, the results clearly show that diffusivity in the normal direction has decreased significantly for this dense sample. Referring to Figure 4.21 for example, it is 30% lower than that parallel to the nanotubes.

Additional information from the structure-diffusivity relationship is given in Figure 4.22, which shows that water diffusivity within CNT decreases as the diffusion time and diffusion length increases.

This data also indicates that after the sample is shrunk, the diffusion anisotropy increases significantly. In order to further explore these effects, the results of Figure 4.11-
4.13 are fitted using bi-exponential function, which corresponds to signal decay according to a two-compartment model. The results are summarized in Table 4.1.

Figure 4.22. Diffusivity decrease as diffusion length increases.
Table 4.1. Diffusivity results of Figure 4.11-4.13. Diffusion normal to CNT axis with diffusion time of 15ms and 30ms are fitted with bi-exponential curve to extract the diffusivity for each compartments from the two-compartment model

<table>
<thead>
<tr>
<th>Δ (ms)</th>
<th>7</th>
<th>15</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parallel ($×10^9 \text{m}^2/\text{s}$)</td>
<td>1.643</td>
<td>1.374</td>
<td>1.342</td>
</tr>
<tr>
<td>Normal ($×10^9 \text{m}^2/\text{s}$)</td>
<td>1.661</td>
<td>1.834 (75-80%)</td>
<td>1.935 (70-75%)</td>
</tr>
</tbody>
</table>

In the two-compartment model introduced in section 4.3, free bulk water is the dominant compartment with higher volume fraction around 80%, and the other compartment (structural compartment) is consider to be filled with CNT structures, like nanotube, ropes, or bundle of ropes. However, as shown in Table 4.1, the diffusivity for the structural compartment, extracted from bi-exponential curve fitting of experiment data, is significantly smaller than the three estimated values ($1.16×10^9 \text{m}^2/\text{s}$, $1.787×10^9 \text{m}^2/\text{s}$, and $1.95×10^9 \text{m}^2/\text{s}$ at different levels). This indicates the film is not organized ideally as it was assumed in the simplistic model of section 4.3. As seen on Figure 4.8 (d), the inter-

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tube space is connected with complex ‘networks’ instead of filled with water. Hence the model discussed in section 4.3 oversimplifies the intertube geometry.

### 4.6 Conclusion

As the first trial to measure water diffusion within VA-CNT membrane, this experiment demonstrates that diffusion-weighted MRI is sensitive to detect the anisotropic diffusion, and explore the underlying structure at some extent. The measured diffusivity can be used to fit in multiple compartment models and explain the signals decay.

At the same time, the accuracy of the measurement needs to be further improved. CNT sample purity, hydration for shrunk sample, and magnetic field homogeneity, partial volume effect are all important factors on the images quality, and on the result interpretation. Also, a strong magnetic field is needed to detect smaller structure, since the slow diffusive motion can only be observed at high b values.

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CHAPTER 5

SIMULATION OF DIFFUSION-WEIGHTED NMR SIGNAL ATTENUATION FROM ELLIPTICAL MYOFIBER

5.1 Introduction

As it was discussed in Chapter 1 and shown in Figure 1.1, human skeletal muscle has a highly hierarchical organization. The emphasis here is on the striated muscle fiber which composes of the functional part of skeletal muscle tissue and excludes fat depots, nerves, and blood vessels. Considering a cross-section through the transverse plane (normal to the muscle fiber direction), a typical skeletal muscle has a diameter of 1-10 cm, and consists of bundles of fascicles, each about 1-2mm in diameter. Each fascicle is a bundle of myofibers, also called myocytes, which are the elementary unit in muscle tissue. Each myofiber (50-100µm) also consists of a bundle of myofibrils. As shown in Figure 5.1, each myofiber is surrounded by the cell membrane (sarcolemma), and is composed of an array of hundreds of parallel myofibrils (1-2 µm) arranged in bundles parallel to the axis of muscle contraction. Finally, each myofibril is composed of a bundle of myofilaments, consisting of a periodic lattice (honeycomb pattern) of the active
contraction pair myosin-actin, tethered by other protein filaments.

Figure 5.1. Micro-anatomical detail of single myofiber (Martini et al. 2011).

The multiple levels of organization discussed above are delineated by various matrix layers which serve to integrate to the muscle components while allowing the muscle to contract. Each myofibril is surrounded by intermediate filaments (desmin and plectin), a longitudinal tubular system called sarcoplasmic reticulum, and a transverse tubular system (T-tubules) which is an invagination of the sarcolemma and communicates with extracellular space. Outside the myofiber, the extracellular space is filled with intramuscular collagenous tissue, also organized in a hierarchical fashion: myofibers are separated by endomysium, fascicles are separated by perimysium, and muscle tissue is surrounding by epimysium. The perimysial network surrounding myofibers forms axial and lateral tethers between the cells (Passerieux et al. 2006).
5.2 Anisotropic diffusion in skeletal muscle transverse plane

The ability to probe local diffusion barriers non-invasively has made Diffusion Tensor Imaging (DTI) a very powerful tool to study the complex muscle microstructures. It has been used to reconstruct the fiber tracts in the calf muscle (Damon et al. 2002, Galban et al. 2004), in the tongue (Wedeen et al. 2001), and in the myocardium (Tseng et al. 2003). Not surprisingly, such heterogeneous and hierarchical structure results in highly anisotropic water diffusion within the muscle. Early NMR measurements (Cleveland et al. 1976) within skeletal muscle have revealed that water diffusivity along the myofibers is significantly higher than that perpendicular to them. With the development of DTI, another level of diffusion anisotropy is revealed on the transverse plane of skeletal muscle, which is defined by the plane perpendicular to the local fiber direction. In prior DTI experiments on skeletal muscle, it has been reported that the apparent diffusion tensor is anisotropic on the transverse plane; the secondary eigenvalue is significantly higher than the tertiary eigenvalue (Damon et al. 2002, Galban et al. 2004, Sinha et al. 2006). Both eigenvalues, which characterize the spin diffusion on the transverse plane, are smaller than the primary eigenvalue.

To explain the anisotropic water diffusion in the muscle, different models have been proposed to explore restricted diffusion and the resulting DTI signal, each attributing the diffusion anisotropy to different mechanisms. Deux et al. (Deux et al. 2008) tried to identify the origin of the transverse anisotropy by monitoring the variations of
eigenvalues while the calf muscle is contracting. Heemskerk et al. (Heemskerk et al. 2008) proposed that this anisotropy is caused by different structures, since the secondary eigenvalue stays constant while the tertiary eigenvalue decreases during muscle extension. Galban et al. (Galban et al. 2004) suggested that the secondary eigenvalue is related to the diffusion process within the endomysium, while the tertiary eigenvalue corresponds to the diffusion process within the individual fibers.

Several histological observations reveal that the cross-sectional geometry of myofiber is of a generally elongated polygonal shape, instead of a regular polygon (Aquín et al. 1980, Campos et al. 2002, Staron et al. 1999). Figure 5.2 shows a typical biopsy image of human vastus lateralis muscle, showing the typical asymmetric myofiber cross sectional geometry. The outline of the myofibers has been approximated by an elliptical shape both for skeletal muscle (Aquín et al. 1980) and the myocardium muscle (Gerdes et al. 1994).

Although no statistical analysis of on the direction of the elliptical shape, a general orientation may exist within a single voxel, and influence the results in DTI experiments.
Inspired by these observations, Karampinos et al. (Karampinos et al. 2009) proposed that the asymmetry of the diffusion tensors on the transverse plane results from the cross-sectional asymmetry of the myocyte. They approximated the skeletal muscle fibers by cylinders of elliptical cross-section. They also used a composite medium model to simulate water diffusion in the space within the muscle fiber and the extracellular space (endomysium). The composite medium model was based on the Kärger bi-compartmental model (Kärger et al. 1988) for water diffusion, which assigns lumped capacitances to this intra- and trans-compartmental mass exchange. This model was originally applied to modeling axons in white matter (Stanisz et al. 1997), the results are consistent with high angular resolution diffusion imaging (HARDI), but the analysis is based on 0-dimension modeling. The model assumes a linear superposition of MRI signal
in the interior and the exterior compartments and a uniform distribution of signal in each of the two compartments. These assumptions require justification when the model is applied in the muscle which has much larger cell (myocyte ~ 50-100μm) than white matter (axons ~ 1-10μm).

The justification of using such composite medium models to model restricted diffusion in myocytes will be provided here by direct numerical simulation of NMR signal. Specifically, we will perform the direct numerical integration of the equation describing the NMR signal in the transverse plane of the muscle using a periodic model consisting of a periodic array of elliptic myofiber embedded in a continuous matrix. The volume-average diffusion-induced signal attenuation will then be used to obtain the anisotropic diffusion coefficients on the transverse plane. This Chapter is organized as follows: In Section 5.3, the composite medium model is reviewed and the simplified 2D myofiber model is presented. Section 5.4 describes the numerical solver for the NMR signal equation (Bloch-Torrey). The governing equations, the numerical algorithm as well as the Lattice Boltzmann (LB) scheme for the diffusion solver are also introduced. Finally, the simulated signal attenuation is presented and diffusion anisotropy is analyzed in section 5.5, and the validity of a simple two-compartment diffusion model is discussed.

5.3 Two-compartment model for muscle fiber

The composited medium model (Karampinos et al. 2009) considers the muscle
fibers as infinite cylinders with an elliptical cross section, as shown in Figure 5.3.

![Diagram of muscle fibers and cross section](image)

Figure 5.3. (a) Simplified muscle fiber model and the transverse plane. The model consisted of infinitely long cylinder, surrounded by the water permeable sarcolemma membrane, and embedded in the extracellular endomysium. (b) Cross section of a single myofiber in a periodic model of muscle. The perimeter of the ellipse consists of a membrane with finite water permeability.

The geometric ratio of the elliptical cross section $\alpha$ (short axis length/long axis length), or ellipticity, is estimated to be in the range 0.40-0.80 (Aquin et al. 1980), based on studies of animal skeletal muscle. It is also assumed that the endomysium microstructure contributes nothing to the diffusion anisotropy, because fibrils in the endomysium are arranged isotropically in the relaxed muscle. Consequently, the muscle fiber has been simplified as a two-compartment system, which enables the connection between the MRI signal and the model parameters of the composite medium. Taking into account the trans-compartmental water exchange, Kärger’s model (Kaerger et al. 1988)
was used to derive an analytical expression for echo attenuation signal, and the results were found to be consistent with measurements (Karampinos et al. 2009).

In the present work, the directional water diffusion the transverse plane is simulated by solving the unsteady diffusion equation for the spins in a 2D elliptical two-compartment medium, as shown in Figure 5.3(b). Based on the results of the previous study (Karampinos et al. 2009), the following geometry and diffusion parameters are used: the muscle fiber is 94 microns in the long axis and 66 microns in the short axis, which gives ellipticity of 0.7. For the intracellular compartment, the diffusion coefficient is assumed to be $1.6 \times 10^{-9} \text{ m}^2/\text{s}$, and the transverse spin-spin relaxation is assumed to be $T_{2, \text{in}} = 30 \text{ms}$. These two properties have values of $2.0 \times 10^{-9} \text{ m}^2/\text{s}$ and $110 \text{ms}$, respectively, for the extracellular space (Karampinos et al. 2009). Because a rectangular computation domain is coincident for the periodic boundary condition imposed, the highest volume fraction that can be reached under this geometry is around 0.7, which is lower than what is reported for histology studies the histology reports. This discrepancy is not important, because we only focus here on proving the validity of the composite-medium model to explain the transverse diffusion anisotropy in muscle, and not to simulate a realistic muscle fiber.

There are different MRI pulse sequences that can be used in diffusion imaging, including Pulse Gradient Spin-Echo (PGSE), Oscillating Gradient Spin-Echo (OGSE) (Gross et al. 1969), Modified Oscillating Gradient Spin-Echo (MOGSE) (Kiruluta 2008),
etc. All these sequences use diffusion gradient pairs to accumulate phase incoherence caused by spin diffusion, collect the echo signal by scanning k-space, and then reconstruct the images from raw data. Since we are only interested in simulating diffusion-attenuated signals, the imaging acquisition and reconstruction parts of the MRI procedure are not simulated. Without imaging gradients, a simple bipolar diffusion gradient pair can be used to detect the diffusion effect, with a pulse gradient duration of $\delta$, and gradient interval $\Delta$ (usually referred to as diffusion time), see Fig 2.3 (b). For a gradient pair of strength $g$, the diffusion-attenuated signal decays exponentially:

$$\frac{S(g)}{S_0} = e^{-bD},$$

where $D$ is the local diffusion coefficient, $b$ is the diffusion weighting given by equation (2.16), $\gamma$ is the gyromagnetic ratio for $^1H$ and $S_0$ is the system signal when no diffusion gradient is applied ($b=0$). In this study, two diffusion directions, along the long and along the short axis of the elliptical cross-section, are investigated. The apparent diffusion coefficient along each direction can be obtained from linear data fitting of the signal decay curve. A diffusion gradient pair with duration of 16 ms, and gradient pulse interval of 40 ms is used in the current simulation, which are typical values used in prior experiments (Karampinos et al. 2009). Typical $b$-values for diffusion MRI experiment on human muscle range in the domain $0\sim500 \, s/mm^2$ (Karampinos et al. 2009), hence only the simulated results under this $b$ value range are used for linear curve fitting. For the simulations, on the other hands, the gradient strength is chosen so as to obtain $b$-values ranging from $0\sim2500 \, s/mm^2$, which enables the investigation of signal
behavior for high b-values.

5.4 Signal simulation algorithm

5.4.1 Governing equation discretization

The governing equation describing spin dynamics in the presence of diffusion during an NMR experiment is the Bloch-Torrey equation (Torrey 1956). In skeletal muscle fiber modeling, diffusion is usually the only mass transport process that needs to be considered, thus the flow term can be neglected. The Bloch-Torrey equation is the extension of equation (2.5) and can be written in the rotating frame as:

\[ \frac{\partial M_\perp}{\partial t} = -i\gamma r \mathbf{g} M_\perp - M_\perp / T_2 + D \nabla^2 M_\perp, \tag{5.1} \]

where \( M_\perp \) is a complex number representing the transverse bulk magnetization (which has two components \( M_x \) and \( M_y \)), and \( T_2 \) is the local spin-spin relaxation time. Put in scalar form and discretized both spatially and temporally, equation (5.1) can be decomposed in three sequential steps:

(a) Diffusion step described by Fickian diffusion of spins in 2-D:

\[ \frac{\partial M_x}{\partial t} = \frac{\partial}{\partial x} (D \frac{\partial M_x}{\partial x}) + \frac{\partial}{\partial y} (D \frac{\partial M_x}{\partial y}) \]
\[ \frac{\partial M_y}{\partial t} = \frac{\partial}{\partial x} (D \frac{\partial M_y}{\partial x}) + \frac{\partial}{\partial y} (D \frac{\partial M_y}{\partial y}) \tag{5.2} \]
where D is spatially varying in the composited medium model.

(b) **Free precession step:**

\[
\Delta \phi = \gamma g(i - N/2) \Delta x \Delta t \\
M_x^{n+1/2}[r] = M_x^n[r]\cos(\Delta \phi) - M_y^n[r]\sin(\Delta \phi) \\
M_y^{n+1/2}[r] = M_y^n[r]\cos(\Delta \phi) + M_x^n[r]\sin(\Delta \phi)
\]

where \( \Delta x, \Delta t \) are the spatial and temporal step size for the discretization, and \( N \) is the spin isochromat numbers along the gradient direction. Without losing generality, the gradient center is placed on the middle of the sample, and then \( \Delta \phi \) represents the accumulated phase during each time step for the \( i \)th spin isochromat.

(c) **The spins relax continuously during the entire gradient pulse:**

\[
M_x^{n+1}[r] = M_x^{n+1/2}[r]\exp(-\Delta t/T_2[r]) \\
M_y^{n+1}[r] = M_y^{n+1/2}[r]\exp(-\Delta t/T_2[r])
\]

where \( r \) is the location of the grid point in the computational domain.

Previous numerical simulations of NMR signal have been implemented the finite different (FD) method to solve diffusion equation (5.2) (Chin et al. 2002, Hwang et al. 2003). The results from up to 2/3 of the computation domain should be discarded because of an artifact introduced by the periodic boundary treatment in FD (Hwang et al. 2003), and hence the computational efficiency is reduced dramatically. This problem gives us
impetus to use another diffusion solver, which is based on the Lattice Boltzmann (LB) Method (LBM).

5.4.2 Code verification and membrane boundary condition

LBM is used to solve the 2-D partial differential equation (5.2) describing the diffusional motion of water spins during the sequence. LBM is a highly efficient and inherently parallelizable mesoscale numerical scheme, which has been introduced in Chapter 2. The governing equations for LBM and the physical meaning of the various parameters were also summarized in Chapter 2. In this section, the LBM implementation is validated and the numerical treatment of the permeable membrane boundaries is introduced for the myofiber model. Equation (5.2) is essentially the unsteady diffusion equation in a heterogeneous medium. To validate the LBM solver, unsteady 1D diffusion along a semi-infinite body and steady-state diffusion through a heterogeneous medium are simulated, and the results are compared with theoretical or empirical formulas.

5.4.2.1 Unsteady diffusion simulation using LBM

The 1D diffusion along a semi-infinite body is described by the equation:

\[
\frac{\partial M}{\partial t} = D \frac{\partial^2 M}{\partial x^2},
\]

Subject to the following initial (5.6) and boundary (5.7) condition:
\[ t = 0, \quad M(x,0) = t_0 \]  
\[ x = 0, \quad M(0,t) = M_w; \quad x \to \infty, \quad M(x,\tau) = M_o, \]  
(5.6)  
(5.7)

the analytical solution can be expressed as:

\[ \frac{M - M_w}{M_o - M_w} = \text{erf} \left( \frac{x}{2\sqrt{Dt}} \right). \]  
(5.8)

LBM is used to solve this problem on a 2000×3 square domain, which can approximate the 1D domain. The parameter D was set at 0.25, \( M_0 = 0 \), and \( M_w = 1 \). Figure 5.4 demonstrates the agreement between simulation results and the analytical solution at different times.

![Figure 5.4](image)

Figure 5.4. Comparison between simulation results and analytical solution. Postfix ‘a’ is for ‘analytical’, and ‘s’ is for ‘simulation’.
5.4.2.2 Simulation of 2D heterogeneous diffusion system

To validate the code for 2D diffusion problem in a heterogeneous medium, we focus on computing of the effective diffusivity of a model medium. The model is composed of square inclusions of diffusivity $D_I$ embedded in a continuous medium (of diffusivity $D_C$). Figure 5.5 shows two versions of the model medium, with inclusion volume fractions of 0.1 and 0.6.

![Figure 5.5. 2D heterogeneous medium. Red block are inclusion embedded in blue continuous medium. Inclusion volume fraction is 0.1 on the left, and 0.6 on the right configuration.](image)

The computed effective diffusivity is compared with the analytical results given by Maxwell’s equation (Alvarez et al. 1996):

$$D_r = \frac{D_{\text{eff}}}{D_c} = \frac{2D_c + D_I - 2\phi(D_c - D_I)}{2D_c + D_I + \phi(D_c - D_I)}, \quad (5.9)$$

where $\phi$ is the volume fraction of the inclusions. As shown in Figure 5.6, the simulation results compare well with theory.
Figure 5.6. Result comparison between LBM simulation and theory (Maxwell’s equation) for diffusion in the two compartment heterogeneous mediums of Figure 5.5. (a) denotes analytical and (s) denotes simulation results.

5.4.2.3 Simulation of permeable membrane as boundary condition in LBM

Myofibers are surrounded by sarcolemma that can be modeled as a water-permeable membrane, which nevertheless constitutes a diffusion barrier that limits water exchange between intracellular and extracellular spaces. Hence a model to describe the water diffusion should take into account the barrier effect of the sarcolemma.

During the application of Lattice Boltzmann method, the implementation of boundary conditions reduces to the problem of expressing the unknown incoming single particle distribution in terms of known outgoing distribution functions (Noble et al. 1995). Referring to Figure 5.7, let us suppose that the membrane lies between two neighboring lattice points. The mass particle crosses the membrane uninhibitedly if it is fully permeable, and bounces back if the membrane is impermeable to the particle. Water
exchange across the sarcolemma membrane obeys neither of these extreme scenarios, and the membrane needs to be assigned a finite permeability.

![Schematic showing typical diffusion lattices model and a membrane lies between two lattice points. Upper right shows the case when membrane is completely permeable and lower right show an impermeable membrane.](image)

In order to quantify this permeability, a probability parameter $P$ is introduced in the LBM boundary condition treatment, which represents the probability of particles crossing the membrane. To model the permeable sarcolemma in the two-compartment model, the above LBM diffusion equation solver incorporating the permeable boundary treatment was used to solve the unsteady diffusion problem in an 1-D domain separated by a permeable membrane at the mid-point. The same problem was then solved using a standard Finite Difference (FD) scheme with the same spatial and temporal resolution of LBM and the following boundary condition: a membrane of permeability $K$ lies between two grid points, defining the compartments as shown in Figure 5.8.
Referring to Figure 5.8, the permeability $K$ is defined by the relations:

$$J = -K(C_{mem1} - C_{mem2}) = -D_1 \frac{C_{mem1} - C_1}{\Delta x / 2} = -D_2 \frac{C_2 - C_{mem2}}{\Delta x / 2}, \quad (5.10)$$

Note that the membrane of finite permeability creates concentration discontinuity across the membrane. By repeating the LBM simulation with parameter $P$, its relation with the membrane permeability $K$ used in FD simulation was obtained, as shown in Figure 5.9. The water permeability of the human sarcolemma has not been reported, so we use a range of permeability values obtained from marine animal experiment (Sorenson 1971). The single permeability value reported by Suleymanina (Suleymanian et al. 1996) of $K=17\mu m/s$ falls within that range.
5.5 Results and discussion

The simulation starts right after the 90° excitation pulse is imposed, when $M_x$ and $M_y$ are initialized on the transverse plane. The signal is obtained at the end of the second pulse gradient, as shown in Figure 2.3 (b) by averaging over the computational domain of Figure 5.3 (b), and divided by $S_0$. For each set of physical cell parameters, the simulation is repeated for six $b$ values by varying the $g$ value, according to equation (2.16). Both the geometry shape of the muscle fiber and the permeability of the sarcolemma, which confines the trans-compartmental water exchange, will contribute to the diffusion anisotropy. The influence of elliptical geometry of the muscle fiber on the diffusion anisotropy is investigated first by assuming that the sarcolemma is completely permeable to water molecules, that is $K \rightarrow \infty$ (no membrane barrier). Referring to Figure 5.3 (b),
Figures 5.10 and 5.11 depict the simulation results for the apparent diffusion coefficients, $D_x$ along x axis and $D_y$ along y direction, for two values of myofiber ellipticity of 0.70 and 0.38. The solid data points represent simulation results, and the curves represent exponential fitting used to extract the apparent diffusion coefficients. In Figure 5.10, the curve fitting results shows that $D_x = 1.7333 \times 10^{-9} \, m^2/s$ and $D_y = 1.6616 \times 10^{-9} \, m^2/s$; only a small difference between the secondary eigenvalue and the tertiary eigenvalue is observed, compared with the reported values in prior study (Karampinos et al. 2009). This small difference between $D_x$ and $D_y$ is reasonable because the membrane does not limit water exchange between the compartments.

![Diagram](image)

Figure 5.10. Diffusion along x and y directions with no sarcolemma membrane barrier and ellipticity 0.7. Curve fitting result: $D_x: y = -1.7333x+0.00192$, $D_y: y = -1.6616x+0.00175$. 

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To study cases where ellipticity differs from 0.7, the fiber geometry is changed based on the model proposed by Trotter et al (Trotter 1991, Trotter et al. 1992) that the lateral area and volume of the myofiber are kept constant when it contracts. These two geometric constraints can be expressed by the following formulas:

\[
\begin{align*}
\frac{a'}{a} &= 1 + \varepsilon \frac{a^2}{a^2 - b^2} \\
\frac{b'}{b} &= 1 - \varepsilon \frac{b^2}{a^2 - b^2} \\
\frac{c'}{c} &= 1 - \varepsilon
\end{align*}
\]

where \( a, b \) are half length of the long and short axis of the elliptical myofiber respectively, \( c \) is the length of the myofiber, \( \varepsilon \) is a small dimensionless number that represents the axial contraction of the myofiber, and the primed variables represents the new values of \( a, b, \) and \( c \) after the contraction. Note that the transversal ellipticity is defined by the ratio \( b/a \).

Using these formulas, when \( a' \) and \( b' \) are set to 68 microns and 26 microns, respectively, the ellipticity can be lowered to 0.38. The NMR signal simulation is performed for the new geometry while the diffusion parameters kept the same as in previous 0.7 ellipticity case. The outer space is chosen as the minimum rectangular computation domain for the myofiber, which results in a volume fraction about 71%, which is very close to the 70% volume fraction in last case. The simulation results are given in Figure 5.10.
Figure 5.11. Diffusion along x direction and y directions with no sarcolemma membrane barrier and ellipticity 0.38. Curve fitting result: Dx: \(y = 1.7049x + 0.00415\), Dy: \(y = 1.6035x + 0.00247\).

To compare the diffusion anisotropy in these two geometries, the following parameter is used, by following the definition of the planar index (Karampinos 2008)

\[
C = \frac{D_x - D_y}{0.5 \times (D_x + D_y)} \times 100\% \tag{5.12}
\]

C is 4.22% for myofiber ellipticity of 0.7 and it increases to 6.13% when the myofiber is shortened which results in an ellipticity of 0.38. This result indicates that as the myofiber becomes more elliptical, the diffusion anisotropy becomes more significant, as expected.
Next, the water permeability of the sarcolemma is taken into account. Sorenson et al (Sorenson 1971) measured the water permeability through the muscle cell membrane in marine crabs, the results shows a two order difference between the so called “diffusion permeability” and the “non-diffusion permeability”. The latter takes into account all of the possible water exchange mechanisms, including both active transport and pure diffusion. The two extreme values are employed to study the influence of sarcolemma as diffusion barrier on apparent diffusion of water. The probability factor P for the LBM boundary treatment is extracted from the results shown in Figure 5.9.

Figure 5.12 shows the simulation for ellipticity 0.7 using non-diffusion (active transport) permeability of 98 \( \mu m/s \), while Figure 5.13 corresponds to the case using the smaller (diffusion) permeability of 1.21 \( \mu m/s \).
Figure 5.12. Diffusion along x and y directions with sarcolemma permeability equal to 98 \( \mu m/s \) and ellipticity 0.7. Curve fitting result: \( D_x: y = -1.6400x + 0.003190 \), \( D_y: y = -1.47301x + 0.00376 \).

Figure 5.13. Diffusion along x and y direction with sarcolemma permeability equals to 1.21 \( \mu m/s \) and ellipticity equals to 0.7. Curve fitting result: for \( D_x: y = -1.5157x + 0.00303 \), for \( D_y: y = -1.1402x + 0.0002 \).
The influence of sarcolemma permeability on the diffusion anisotropy can be clearly seen from Figures 5.12 and 5.13. As the membrane becomes less permeable to water exchange between the two compartments, the apparent diffusion coefficients ($D_x$ and $D_y$) along both directions decrease significantly. When the permeability decreases, the effect of the geometrical anisotropy of the muscle fiber on diffusion anisotropy becomes stronger.

Turing our attention to the behavior of the diffusion-weighted signal with the diffusion decay factor $b$, we examine the agreement of the simulation with the simple model given by equation (2.17). Under relatively high $b$-values, the simulated signal is observed to deviate from the mono-exponential diffusion curve. A bi-exponential behavior becomes rather more prevalent as shown in Figure 5.14. This is typical behavior of MRI signal for compositéd medium with more than one compartment of spins.

Figure 5.14. Bi-exponential signal decay with sarcolemma permeability equals to 98 $\mu m/s$
We can see that in our two-compartment model with membrane shown in Figure 5.3(b), the anisotropic structure includes both the inhomogeneity in compartment properties (diffusivity, relaxation times) and the surrounding diffusion barrier. Such structures cause the diffusion to deviate from mono-exponential signal decay. This behavior is consistent with the two-compartment model by Kärger (Kärger et al. 1988).

The last question is whether there are conditions where the Kärger two-compartment model which assigns lumped capacitances for each compartment is valid. To establish that, we plot the spatial distribution of signal in the computational domain, the result is given in Figure 5.15. As seen on Figure 5.15, the NMR signal has sharp changes on the compartment boundary, but uniform profile within each compartments, hence the simple two-compartment model is valid.

![Figure 5.15. Signal distribution in the computation domain (along the long axis of the myofiber) shows sharp changes on the compartment boundary and uniform profile within each compartment.](image)

Figure 5.15. Signal distribution in the computation domain (along the long axis of the myofiber) shows sharp changes on the compartment boundary and uniform profile within each compartment.
5.6 Conclusion

The diffusion-induced signal attenuation from an elliptical myofiber is obtained by numerical simulations. The muscle fiber is modeled using the composite medium model proposed in (Karampinos et al. 2009), consisting of two compartments and a myofiber surrounded by a finitely permeable sarcolemma. The simulation results show the typical exponential decay under low b-values and the bi-exponential decay under higher b-values. The resulting apparent diffusion coefficients along different directions of the transverse plane show the anticipated anisotropy behavior. This anisotropy is greatly influenced by the water permeability of the surrounding sarcolemma, and the degree of ellipticity of the cross-section of the myofiber. By simulating the NMR signal decay directly, the applicability of the composited medium to explain the diffusion anisotropy of skeletal muscle on the transverse plane is verified. The potential of LBM scheme to solve the diffusion equation on highly complex geometries will enable us to simulate signal attenuation based on realistic muscle histology data.

5.7 References


CHAPTER 6

CONCLUSION AND FUTURE WORK

6.1 Summary

This work focuses on studying the effect of mobility barriers on water diffusion in heterogeneous media via MRI. Diffusion-weighted imaging has been applied to probe porous materials consisting of a network or fibrous matrix with a short-range order, i.e. exhibiting periodicity within the imaging voxel. Both experiments and theory are used to extract the apparent water diffusion coefficient from the MRI signal decay induced by restricted diffusion in the media.

Motivated by the application of MRI to study water transport in skeletal muscles, three types of engineered or biological structures are discussed: swelling hydrogels, aligned carbon nanotube (CNT) membranes, and a composite medium model for the myofiber.

In chapter 3, the purpose of the MRI experiment is to explore the relationship between water diffusivity and water content of a Polyethylene Glycol Diacrylate (PEG-DA) hydrogel material. The polymer network scaffold of the hydrogel varies with the water concentration, and hydrogel swelling involves a dynamic balance between internal
stresses and intermolecular forces. MRI was used to acquire co-registered proton-density-weighted and diffusion-weighted images of the hydrogel gels.

To quantify the relationship between water diffusivity and water content, two types of gel sample were prepared and used in the experiment: the first with uniform water content used for validation of the measurement technique, and the second with a water concentration gradient allowing a wide range of water contents. The second type of samples was prepared by controlling the diameter of the dry samples and swelling them in same size glass tube. These results can be used to model the swelling dynamics of PEG-DA hydrogel material, and to explore the motion of water in contracting skeletal muscle.

Chapter 4 focuses on water diffusion within vertically aligned CNT films, and specifically in the inter-tube space created by hierarchical structure of the as-produced film with and without shrinkage. The study aims to quantify diffusion so as to aid in developing better CNT membranes by filling the inter-tube space, and to use the CNT bundles as a phantom for myofibril structures in the myofiber of skeletal muscle. A microcoil was used to acquire diffusion-weighted images for variable diffusion times, corresponding to spatial scales in the micrometer range. The impurity residues on the CNT film, magnetic susceptibility artifacts, and eddy currents limit the SNR and confound somewhat the extraction of the diffusion coefficient.

The basic question is at what solid fraction the aligned nanotubes create diffusion anisotropy. The statistical analysis of the result reveals that water diffusivity normal to
the nanotube direction is about 13% lower than that parallel to nanotubes; and diffusivity in the parallel direction is also 20% lower than bulk water free diffusion. The results demonstrate that the aligned CNT structure induces detectable diffusion anisotropy at a volume fraction of about 20%. Since the volume fraction of myofibrils in skeletal muscle is much higher than this value, they have a stronger influence in creating diffusion anisotropy in muscle MRI measurements. Curve fitting results with a bi-exponential formula reveal that diffusivity normal to CNT axis is much lower than simulation results. Such results might partially due to the fact that the interstitial space within the CNT film contains multiple scales.

In chapter 5, numerical simulation is used to predict the diffusion-weighted NMR signal from an elliptical myofiber in a periodic array configuration, surrounded by a uniform matrix representing the endomysium, and with the sarcolemma represented by a partially permeable diffusion barrier. The numerical code integrates the Bloch-Torrey equation in 2-D using the Lattice Boltzmann method. The results demonstrate that the diffusion-weighted signal within each compartment is uniform, and that the NMR signal can be considered as a superposition of the signal from the individual compartments, which agrees with a simplified two-compartment model by Kärger. Furthermore, the calculated apparent diffusion coefficient is anisotropic, as have been observed in experiments. Hence the simulation directly supports the explanation that anisotropic diffusion in the myofiber transverse plane could be induced by the elliptical shape of the
myofiber. More importantly, it also reveals that the barrier effect of sarcolemma is more dominant than the geometry itself on influencing the diffusion anisotropy.

6.2 Future work

The general problem of extracting geometrical information encoded by diffusive motion of molecules (like water) in heterogeneous media from MRI is very complex. Even the forward problem is unsolved, let alone the inverse. This is due to two difficulties: (i) the complexity of the diffusion barriers, (ii) the statistical averaging involved in the MRI technique. The first issue is somewhat mitigated by the presence of short range structural order in the voxel. The second requires improved MRI methodology.

For the measurement of water diffusivity in PEG-DA hydrogel, more data in the low water content regime should be obtained to improve the correlation with the water contents. Since the exponential diffusivity model (equation (3.6)) is adopted, the curve fitting results of Figure 3.14 and 3.15 is sensitive to low water content data. The difficulty to obtain these data, especially when using samples with water concentration gradients, lies in the quick diffusion process and long acquisition time required in the particular MRI sequence used. As shown in Figure 3.16, the diffusion homogenizes the water profile in the sample within 2-3 hours. Hence a faster sequence should be used to scan the gel samples to obtain low water content data.
The technical challenge for the CNT experiment lies both in the sample quality and MRI hardware. First, impurity residues on the CNT film should be further removed by chemical cleaning to decrease magnetic susceptibility artifacts. The nanotubes should be better aligned by growing shorter nanotubes. Long CNT tends to tangle with each other forming network and reduces the structure anisotropy. However, shorter CNT film also means smaller sample size decreased MRI SNR. A compromise should be made between CNT synthesis and MRI imaging requirement. On the experiment hardware aspect, a more powerful gradient and better RF coil is needed to improve the imaging quality. Powerful gradients will enable to detection of slower diffusion.

On the modeling of myofiber, the flexibility and versatility of the Lattice Boltzmann Method enables the extension of the model to more complex biophysical systems. For example, the model can be extended to 3-D and to account for the effect of intra-myocellular fat on water diffusion within each myofiber. Such models can ultimately be used to model water diffusion in the full muscle, and provide a platform to numerical simulation of Diffusion Tensor Imaging signal.