FEASIBILITY STUDY OF MULTI-APPLICATION, MULTI-WALLED CARBON NANOTUBES FOR MAGNETIC RESONANCE TEMPERATURE IMAGING GUIDED LASER INDUCED THERMAL THERAPY

By

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LIST OF ABBREVIATIONS

\( B_0 \) – Main Magnetic Field Strength

\( B_1 \) – Radio Frequency Field Strength

\( B_{\text{nuc}} \) – The local Magnetic Field Strength

CA – Contrast Agents

CT – Computed Tomography

FERIDEX I.V.® ferumoxides injectable solution

FID – Free Induction Decay

FLASH – Fast Low-Angle Shot

FOV – Field of View

FT – Fourier Transform

Gd-DTPA – Gadolinium-Diethylenetriaminepentaacetic Acid

\(^1\text{H} \) – Hydrogen (or Proton)

LITT – Laser Induced Thermal Therapy

MAGNEVIST® - gadopentetate dimeglumine

MR – Magnetic Resonance

MRI – Magnetic Resonance Imaging

MRTI-guided-MWCNTs-LITT – Magnetic Resonance Imaging Guided Laser Induced Thermal Therapy with Multi-Walled Carbon Nanotubes
MWCNTs – Multi-Walled Carbon Nanotubes

NEX - number of images averaged

Nd:YAG - neodymium-doped yttrium aluminum garnet

NIR – Near Infrared Radiation

RF – Radio Frequency

ROI – Region of Interest

SAI – Small Animal Instruments

SE – Spin Echo

SNR – Signal-to-Noise Ratio

SWCNTs – Single-Walled Carbon Nanotubes

T – Tesla

TE – Echo Time

TR – Repetition Time
ABSTRACT

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FEASIBILITY STUDY OF MULTI-APPLICATION, MULTI-WALLED CARBON NANOTUBES FOR MAGNETIC RESONANCE TEMPERATURE IMAGING GUIDED LASER INDUCED THERMAL THERAPY

Thesis under the direction of
J. Daniel Bourland, Ph.D., Associate Professor of Radiation Oncology

In this work multi-application, multi-walled carbon nanotubes (MWCNTs) as super MR contrast agents and Near-Infrared Radiation (NIR) laser absorbers are combined with Proton Resonance Frequency (PRF) based Magnetic Resonance Temperature Imaging (MRTI) to improve the safety and efficacy of Laser Induced Thermal Therapy (LITT). Instilled MWCNTs enable precise tumor localization and killing of the tumor through preferential high temperature while protecting surrounding healthy tissue by monitoring the 3D temperature distribution.

As advanced MR contrast agents (CA), Fe-containing MWCNTs produced by chemical vapor deposition (CVD) with 600mg Ferrocene, show up to 5 times greater efficiency in changing T2 relaxation properties compared to the clinical MR CA, Feridex. Moreover, MWCNTs behave as super efficient dipole antennae and have a strong NIR absorbance, giving them potential use as a super heating generator in thermal ablation therapy. In this investigation MRTI-guided-MWCNTs-LITT was first evaluated using 3 tissue equivalent gel phantoms: alginate-only, MWCNTs-instilled and MWCNTs-implanted (sub-surface, simulating a subcutaneous tumor containing MWCNTs). For in vivo performance observations, 4 RENCA kidney tumors in their right flanks for four mice were thermally treated using an external laser beam after direct MWCNTs injection, and monitored by MRTI throughout the treatment. MRTI-guided-MWCNTs-LITT in phantom and in vivo experiments were performed using MR-compatible laser systems (fiber-optic and external laser beam) in a 7T MRI small animal scanner (Bruker Biospin). The 3D...
MRTI at 7T field strength provides high temporal resolution with reasonable special resolution, as well as accurate 3-D volume temperature measurement. Phantom results show that the MWCNTs-instilled phantom heated preferentially. During minimally-invasive fiber-optic laser heating (ø 0.6mm, 1 min @ 0.1W), (from 20°C to 47°C; Δt=+27°C), compared to the alginate-only phantom (from 20°C to 25°C; Δt=+5°C). With external non-invasive laser heating (ø 10mm, 0.5 min @ 1.8W), the implanted region of the MWCNTs-implanted phantom showed significantly elevated temperatures compared to the nearby alginate-only medium (Δt=+15°C). Similar temperature differentials were observed in vivo for the implanted RENCA kidney flank tumors with and without (with: maximum of 77°C, Δt=+51°C without: maximum of 44°C, Δt=+18°C) after a single 30s 3W/cm² non-invasive laser irradiation. At two weeks post-treatment, a complete response was observed for flank tumors with MWCNTs + laser treatment, while no response was observed for flank tumors in two control groups that received no MWCNTs + laser or no MWCNTs and no laser. This investigation shows the successful combination of MWCNT and MRTI both in phantoms and in vivo for a small, pre-clinical study using flank tumors in mice. The in vivo results show significant improvement in tumor response for LITT with instilled MWCNTs, therefore, this technology shows feasibility and may be applicable for treatment of superficial tumors in humans.
CHAPTER ONE

INTRODUCTION

1.1 Overview and Objective

New approaches for cancer therapy continue to be developed. Laser Induced Thermal Therapy (LITT) has been investigated as one of several thermal therapies that destroy tumor cells with minimum invasiveness by heating the cell to 55 °C or higher [1-9]. As a result, protein denaturation, membrane lysis, irreversible cell damage, and coagulative necrosis occur in the target area, resulting in cell death. However, several problems limit minimally-invasive or non-invasive LITT from clinical application: 1) the lack of precise definition for the size or shape of the target (a common problem to all local cancer treatments), 2) the complexity of heat transfer properties in different tissues, and 3) difficulties in the control of the laser energy applied, to maximize energy deposition in the target and minimize energy deposition in normal, healthy tissue to prevent morbidity. Without real-time temperature monitoring or effective heat generating agents, clinical use will be difficult due to the low penetration of laser energy, and the high risk of morbidity from uncontrolled energy absorption and heating of surrounding health tissue which could cause irreversible healthy tissues.
One approach to address these problems is to use an advanced contrast agent (CA) for locating the tumor that would also work as a sensitizing agent by coupling the incident laser radiation to increase the local heating while protecting the surrounding healthy tissue from burns [8-12]. Multi-walled carbon nanotubes (MWCNTs) are an ideal candidate for this multi-functional material. Since its discovery in 1991 [13], the nanotube is widely used in industry, material science, and nano-medicine based on its unique and superior physical, mechanical, electronic and optical properties. MWCNTs are produced via high temperature Chemical Vapour Deposition (CVD) using Ferrocene dissolved in xylene is used as a precursor. These Fe containing MWCNTs are hollow structures with an iron core. The multi layers of carbon have diameters ranging from 10-50 nm and length about 1000 nm. These super paramagnetic (with unpaired electrons) nano materials generate large lattice magnetic fields which greatly shorten the $T_1$ and $T_2$ of any nearby water protons indicating that Fe containing MWCNTs should be effective MR contrast agents to label and locate the target area. Here, we explore the $T_1$ and $T_2$ relaxation properties of MWCNTs compared to the clinical contrast agent, Feridex I.V.® (ferumoxides).

MWCNTs have a strong optical absorbance in the NIR region. According to Jackson’s classical antenna theory [14], to couple the light efficiently, the length of the nanotubes should be comparable to half of the wavelength of the incident radiation. With a classical antenna length of about 1μm, MWCNTs behave as a super efficient dipole antennae for NIR wavelength. Our group has demonstrated that nitrogen-doped MWCNTs in media with a small NIR dose can generate a significant amount of heat [15]. In 2009, we reported the long term survival of kidney tumor mice followed by MWCNTs-LITT by using its novel properties [16].

Magnetic resonance imaging (MRI) is a relatively new and important imaging technique for diagnosis and radiation treatment planning. Compared to other imaging techniques such as CT and Ultrasound, MRI provides excellent soft tissue contrast due to the unique proton relaxation properties of different tissues. The MR signal intensity
depends on three important intrinsic tissue factors: the proton density, the longitudinal relaxation time, $T_1$, and the transverse relaxation time, $T_2$. Clinical MR contrast agents like Feridex® and MAGNEVIST® (gadopentetate dimeglumine) are used to enhance the image contrast and improve the detection of tumors by changing the $T_1$ and $T_2$ relaxation properties of nearby water protons. Here we have studied the feasibility of using Fe containing MWCNTs as an effective MR contrast agent. Also MRI is the most versatile and sophisticated imaging technique that includes advanced imaging techniques such as MR Diffusion Tensor Imaging (DTI), MR Spectroscopy (MRS), and MR Temperature Imaging (MRTI). In particular, MRTI exploits the sensitive temperature dependency of the proton’s resonance frequency. [17]. With MRTI, relative temperature maps can be created from measuring the phase change between two MR images. 3D MRTI during LITT provides (i) enhanced safety by visualizing temperature maps around the target area so that healthy tissue can be protected from damage caused by overheating, and (ii) non-invasive real-time temperature monitoring combined with non-invasive thermal therapy. In our work, MRI is the perfect tool for non-invasive LITT for both structure imaging (locating the intended target volume) and temperature monitoring (providing real-time temperature mapping during the treatment).

The ultimate objective of this study was to explore a new efficient, safe and fast MRTI guided LITT with MWCNTs used as multi-functional agents: 1) as MR contrast agents and 2) NIR absorbing agents. A high field strength MRI unit (7T Bruke Biospin) was used in this study to provide high spatial and temporal resolution and high SNR images. Phantom studies were first conducted followed by the use of kidney tumor bearing nude mice for an in vivo tumor regression study.
1.2 Thesis Outline

This thesis includes six individual chapters. Each provides background knowledge and review articles related to the project or the experimental design and results.

Chapter 2 is an introduction to Magnetic Resonance Imaging and relaxation properties. Longitudinal and transverse relaxation time properties and T1/T2 weighted imaging are reviewed relevant to the use of MWCNTs as MR contrast agents and laser absorbers.

Chapter 3 introduces the advanced MRI techniques used in the study: Keyhole imaging technique to increase temporal and spatial resolution, and MR pulse sequences for Magnetic Resonance Temperature Imaging which is used for non-invasive 3D temperature distribution measurement for Laser Induced Thermal Therapy (LITT) both in vivo and with phantoms.

Chapter 4 covers basic knowledge of the synthesis of different Multi-Walled Carbon Nanotubes and their novel properties and application in research frontier. It includes the experiments of using MWCNTs as an effective T2 weighted MR contrast agent in vivo as laser absorbers and heat generators in water solutions.

Chapter 5 covers MRTI guided MWCNT- LITT experiments in vitro (phantom) and in vivo (mouse flank tumors). The MR compatible fiber and external laser system setup for the 7T MR animal scanner is described. Detailed methods and results of the new thermal therapy in vivo are included and significant findings are reviewed.

Chapter 6 gives the discussion and conclusions from all the experiments performed in this thesis. Both MRTI artifacts and limitations and MWCNTs solution stability and toxicity are discussed.
CHAPTER TWO

PHYSICAL PRINCIPLES OF MAGNETIC RESONANCE IMAGING

2.1 Introduction

The concept of Nuclear Magnetic Resonance (NMR) was found by Rabi et al. [91] in the 1930's. With this foundation, Bloch et al. [92] and Purcell et al. [93] began investigating an effect of the precession of the spins in a magnetic field. The famous Bloch equation paved the road for the powerful 3D imaging technique—Magnetic Resonance Imaging (MRI). Both Dr. Lauterbur [94] and Dr. Mansfield [95] received the Nobel Prize in Medicine (2003) for their contribution of obtaining the first images based on NMR and first human body MRI cross section. Compared to other imaging modality techniques like Computational Tomography, Ultrasound, etc, MRI offers noninvasive high resolution, high contrast images of soft tissue structures in the human body without the use of ionizing radiation. As a result, MRI is a popular imaging modality for both clinicians and researchers. There are about 3000 MRI units located in the United States and 20,000 all over the world.
This chapter covers general MRI physics principles, relaxation properties and imaging techniques relative to this project. More detailed or specific topics of MRI techniques can be found. Here, two websites and two text books are recommended. The website www.e-mri.org is a very good introduction for beginners. It explains MRI physics principles in an interesting and simple way without equations including animation tools to simulate K-Space reconstruction, $T_1$ and $T_2$ weighted contrast image acquisition and MRI unit operation. A second website, www.revisMRI.com, has excellent Q&A sections which cover most of the fundamentals of MRI. *MRI Physical Principles and Sequence Design* by Haacke et al. [97] covers classical and quantum mechanical principles, signal detection and acquisition, image artifacts, image reconstruction methods, $k$-space trajectories, flow issues, and MR equipment issues; Another reference book, *Handbook of MRI Pulse Sequence* [98] is an advanced, in-depth treatise of MRI theory. It is a first choice for scientists and engineers who have fundamental knowledge of MRI physics and pulse sequence design.

### 2.2 Nuclear Spin and the Magnetic Moment

The fundamental basis of MRI is the nuclear spin. Spin is an intrinsic property of elementary particles. It is a very abstract concept. However, proton spin can be thought of hydrogen nuclear precessing along the z axis similar to a circulating electric current and it’s associated magnetic moment (Fig. 2.2.1.a). The sum of all the tiny magnetic moments of each spin is called the net magnetization or macroscopic magnetization. Normally, such as in a bottle of water, the proton spins point in random directions. Without an external magnetic field the sum of the random spin is equal to zero, or in other words, null net magnetization. When a strong magnetic field is applied, some of the spins
align with the field (parallel) and some align against the field (anti-parallel). Nuclear spin will "wander" towards an orientation with a lower magnetic energy (parallel) than an orientation with a higher magnetic energy (anti-parallel). Over time, a net magnetization forms. (Fig. 2.2.1.c)

**Figure 2.2.1** (a) Hydrogen nuclei (protons) or nuclear spin behaves like tiny rotating magnets, represented by vectors. (b) Normally, the direction of nuclear spins is randomly distributed. Thus, the sum of all the spins gives a null net magnetization. (c) Within a large external magnetic field, B0, spins aligned with the field with a net magnetic moment along the longitudinal direction. (www.e-mri.org)

The equation to describe the magnetic moment $\mu$ is

$$\mu = \gamma \hbar I$$

(2.2.1)

where $\gamma$ is the gyromagnetic ratio, $\hbar$ is the Planck’s constant ($6.606 \times 10^{-34}$ J) and $I$ is the angular momentum. In clinical imaging, the most commonly studied nucleus is $^1$H because the human body is mainly composed of water. $^{13}$C, $^{19}$F, $^{23}$Na and $^{31}$P are other nuclei with magnetic moments that can be used for biomedical research as well.

A wobbling of spin about the axis of the $B_0$ field is called precession (Fig.2.2.2). Spinning precession corresponds to the gyration of the rotating axis of a spinning body about an intersecting axis. For a particular magnetic field strength $B_0$, the Resonance frequency $\omega_0$ of precession can be calculated according to the Larmor equation.

$$\omega_0 = \gamma B_0$$

(2.2.2)

where $\gamma$ is the gyromagnetic ratio, and $B_0$ is external magnetic field strength.
This equation is fundamental to MRI physics. In MRI, resonance corresponds to the energetic interaction between spins and electromagnetic radiofrequency (RF). Protons that precess with the same, $\omega_0$, frequency as the electromagnetic RF pulse will respond to that RF pulse.

### 2.3 Radiofrequency Excitation

The energy absorption of a photon by radiofrequency (RF) excitation only occurs at the resonance frequency ($\omega_0$). RF excitation changes energy levels and spin phases. Magnetic momentum M can be split into z-axis, (longitudinal $M_z$) and xy-plane (transverse $M_{xy}$) components. Without RF excitation, the proton spin has net magnetization $M_0 = M_z$.

When a spin is excited by a 90 degree RF pulse, the net magnetization vector from z direction tips down to the xy-plane (transverse plan). The vector component $M_{xy}$ creates an oscillating magnetic field and induces a signal in the detection equipment (Fig. 2.3.1). The flip angle is determined by the strength and duration of the RF.
Figure 2.3.1  a signal detector measures the component of magnetic momentum $M_0$ which is tipped down on the $xy$-plane, $M_{xy}$. The flip angle of the $M_0$ depends on the strength and the duration of $B_1$ (RF) ([www.e-mri.org](http://www.e-mri.org))

2.4 T1 and T2 relaxation

After the excitation of the RF, the process in which the net magnetization returns to its original equilibrium position $M_0$ is called Relaxation. Relaxation combines by two different mechanisms: First longitudinal relaxation corresponds to longitudinal magnetization recovery ($T_1$ relaxation); Second transverse relaxation corresponds to transverse magnetization decay ($T_2$ relaxation). Both relaxations are the intrinsic properties of tissue and its surroundings and are described by the phenomenological Bloch equations:

\[
\frac{dM_z}{dt} = -\frac{M_z - M_0}{T_1} \quad \text{Longitudinal relaxation} \quad (2.4.1)
\]

\[
\frac{dM_{x,y}}{dt} = -\frac{M_{x,y}}{T_2} \quad \text{Transverse relaxation} \quad (2.4.2)
\]
Longitudinal relaxation is due to energy exchange between the spins and surrounding lattice (spin-lattice relaxation, $T_1$), coming back to thermal equilibrium state. RF energy is released back into the surrounding lattice, as spins go from a high energy state back to a low energy state. The recovery of longitudinal magnetization follows an exponential curve. The recovery rate is characterized by the tissue-specific time constant $T_1$. After time $T_1$, longitudinal magnetization has returned to 63% of its final value. With a 1.5 T field strength, $T_1$ values are about 200 to 3000 ms. $T_1$ values are longer at higher field strengths.

**Figure 2.4.1** $T_1$ relaxation (spin-lattice relaxation) $M_0$ returns to thermal equilibrium position along z-axis. (www.e-mri.org).

Transverse relaxation is due to magnetic field interaction (spin-spin interaction, $T_2$), which changes spins’ Mxy precession rates. Spin-spin relaxation causes a cumulative loss in phase resulting in transverse magnetization decay. Transverse magnetization decay is described by an exponential curve, characterized by the time constant $T_2$. After time $T_2$, transverse magnetization has lost 63% of its original value. $T_2$ is tissue-specific and is always shorter than $T_1$. Transverse relaxation is faster than longitudinal relaxation. $T_2$ values are unrelated to field strength.
There are three most commonly acquired MRI image types: $T_1$-weighted, $T_2$-weighted and proton density weighted. By changing the pulse sequence parameters, like the echo time (TE) and repetition time (TR), the same image with different contrast can be generated according to the $T_1$ and $T_2$ differences between tissues. The basic expression for image contrast is described as:

$$C_{AB} = \text{SIGNAL}_A - \text{SIGNAL}_B$$  \hspace{1cm} (2.4.3)

In this way it is possible to distinguish between different types of tissue (white and gray matter and cerebrospinal fluid in the brain (Fig. 2.4.3), to detect and delineate pathological tissue alterations which occur for instance in tumors, and to measure many properties of tissue both qualitatively and quantitatively, such as blood perfusion, oxygen concentration, blood throughput of the heart, diffusion, and the concentration of different metabolites. MRI techniques therefore play a key role in medical diagnostics today.
Figure 2.4.3 MR image slices through the head of a patient with brain tumor. (a) T1 weighted image with enhancement of the blood vessels which appear very bright; (b) T2 weighted image which is very useful for showing pathological abnormalities. Grey (G) and white (W) matter of the brain and cerebrospinal fluid (CSF) (F) can be easily distinguished, in particular in the T1 and T2 image.
CHAPTER THREE

MAGNETIC RESONANCE TEMPERATURE IMAGING

3.1 Introduction

Laser Induced Thermal therapy (LITT) is limited due to the difficulty of predicting a precise lesion size or temperature distribution at a certain power output and duration with the different absorption, and attenuation coefficients of the different kinds of tissues. Therefore image guidance is important for LITT for avoidance of undesired damage to the adjacent healthy structures. In recent years, Magnetic Resonance Temperature Imaging (MRTI) guided thermal therapy has been investigated to improve the safety and efficacy of benign and malignant diseases treatment. It is a noninvasive 3D temperature imaging technique to measure the real-time temperature change in the target area. The first report of temperature mapping by MRI appeared in 1983 [98]. Temperature could be calculated from sensitive parameters such as the proton resonance frequency (PRF) [99], T₁ and T₂ relaxation times [98], magnetization transfer [100], the proton density [101] and diffusion coefficient [102] in which RRF based MRTI became more and more popular because of its high temporal resolution and accurate temperature measurement. The
advantage of MRTI is that it not only measures the real-time 3D temperature distribution, but also can be used for precise target localization and early evaluation of thermal therapy.

Several different methods such as Radiofrequency (RF), Focused ultrasound (FUS) and Laser sources can be used to deliver the heat energy for thermal therapy. Focused ultrasound is a noninvasive therapy that uses a crystal transducer to focus the ultrasound to the tumor. Radiofrequency ablation uses an interstitial electrode to deliver the energy through alternating current into the tissue, but the electrode is not compatible with MRI and will cause susceptibility artifacts in the images [20]. LITT uses a laser fiber or external laser beam to deposit energy into the target. Because fiber optic laser delivery is MR compatible and MWCNTs have a high absorption coefficient at wavelength of 1064nm, LITT is an ideal candidate as the energy source for MRI-guided thermal therapy. Here we used a 7T MR unit (Bruker Biospin) and PRF based MRTI technique to monitor the 3D temperature maps in vivo during treatment.

3.2 Proton Resonance Frequency based Magnetic Resonance Temperature Imaging

Hindman first observed the phenomenon of temperature dependent proton resonance frequency (PRF) in 1966 [21]. First MR temperature imaging based on the PRF was proposed by Ishihara et al. [22] and developed by de Poorter and colleagues [23,24]. In water molecules, $^1$H, the hydrogen nuclei, is screened from the external field ($B_0$) by the electrons. The nature of the hydrogen bonds varies with temperature [25]. As the temperature increases, the hydrogen bonds change state, leading to distortion in the electronic configuration of screening. As a result, there is more electron screening of the
hydrogen nucleus which lowers the local magnetic field ($B_{\text{nuc}}$) and also the resonance frequency. It is found that the temperature dependent frequency varies linearly as a function of temperature. For pure water the constant is $1.03 \times 10^{-8}$ °C over the range from 15 °C to 100°C. Detailed principles can be found in two references. [21,26].

Here we will briefly discuss the PRF based MR Temperature Imaging calculation. The local magnetic field ($B_{\text{nuc}}$) can be expressed as a function of the external magnetic field $B_0$:

$$B_{\text{nuc}}(T) = \left(1 - \sigma(T) - \frac{2\chi}{3}\right)B_0.$$  \hspace{1cm} (3.2.1)

where $T$ is the temperature, $\sigma(T)$ is temperature dependent chemical shift field and $\chi$ is the bulk magnetic susceptibility. The chemical shift field (in ppm) is the sum of the temperature independent contributions $\sigma_0$ and temperature dependent contribution, $\sigma_T(T)$:

$$\sigma(T) = \sigma_0 + \sigma_T(T).$$  \hspace{1cm} (3.2.2)

The chemical shift field can be calculated from the phase information in RF-spoiled gradient echo images:

$$\Phi(T) = \gamma\sigma(T)T_EB_0.$$  \hspace{1cm} (3.2.3)

where $\varphi$ is the phase of the image, $\gamma$ is the gyromagnetic ratio ($42.58 \times 10^6$ Hz T$^{-1}$ for protons) and $TE$ is the echo time. To calculate the temperature dependent phase difference, the typical method is to subtract the phase of the images from a reference MR
images where the absolute temperature is known (Fig.3.2.1), eliminating, the term $\sigma_0$. As a result the change in temperature is found as a difference of phases:

\[ \Delta T = T - T_{\text{ref}} = \frac{\Phi(T) - \Phi(T_{\text{ref}})}{\alpha \gamma T_E B_0} \]  

(3.2.4)

where $\alpha=(d \sigma /dT )$ is the temperature dependency of the water chemical shift in ppm C$^{-1}$. PRF-based MR Temperature Imaging can be obtained by any gradient echo sequence, as long as contributions from stimulated echoes can be neglected [27,28]. Spin-echo sequences cannot be used since the temperature induced phase difference will be refocused, which means that signal would not have a phase difference. RF spoiling of fast gradient echoes is, thus, necessary when flip angles close to the Ernst angle are used for optimal SNR for short TR [103, 104].
3.3 MR Pulse sequence

Each MR pulse sequence is a combination of radiofrequency pulses and gradients. There are over a hundred different pulse sequences for different imaging purposes. For example, some sequences favor a particular tissue contrast, temporal resolution (speed), spatial resolution, and/or increase the signal to noise ratio (SNR). The most commonly used pulse sequences are the spin-echo and gradient echo sequence. The three primary parameters of pulse sequences are the repetition time (TR), the echo time (TE) and flip angle (FA). The spin pulse sequence timing can be adjusted to give $T_1$-weighted, proton or spin density, and $T_2$-weighted images. Multi-echo sequences can be used to obtain both proton density and $T_2$-weighted images simultaneously. Besides TE, TR and FA, the gradient echo sequences acquired a basic sequence varied by adding dephasing or rephasing gradients at the end of the sequence instead of an 180 degree refocused pulse in spin echo sequence. The flip angle is usually over a range from 10 to 80 degrees. In this project, we are using gradient echo sequence fast low-angle shot pulse sequence (FLASH), illustrated below (Fig. 3.3.1), to measure phase differences for conversion into MR Temperature Images. This sequence allows for fast 3D imaging during short apnea (10 to 20 seconds) and provides high temporal resolution temperature measurements. This type of sequence is also used in intra-voxel water-fat mixture imaging, by choosing in phase and out of phase TEs (cf. Chemical shift artifact of the second type). The disadvantage of this pulse sequence is its high sensitivity to magnetic susceptibility artifacts.
Figure 3.3.1 Fast Low-Angle Shot pulse sequence (FLASH) gradient pulse sequence schematic. RF with low flip angle tips down the magnetization moment along z direction (Mz). Then followed by a readout gradient (Gfe) to refocus the signal. After a repetition time (TR), a new cycle of FLASH sequence follows. The multi-MR images with temperature dependent phase information are then obtained. (www.e-mri.org)

3.4 K-Space

Raw MR signal data are written into a matrix called K-space. K-space is also called the Fourier plane or frequency domain. Normally, an MR image is the reconstruction from the K-space (frequency domain) to spatial domain using inverse Fourier Transform (FT⁻¹). Fig. 3.4.1 illustrates the 1D Fourier transformations and its signal in time domain. The Fourier Transform (equ. 3.4.1) and its inverse form (3.4.2) are shown below:

\[
\hat{f}(\xi) = \int_{-\infty}^{\infty} f(x) e^{-2\pi i x \xi} \, dx
\]

(3.4.1)

\[
f(x) = \int_{-\infty}^{\infty} \hat{f}(\xi) e^{2\pi i x \xi} \, d\xi
\]

(3.4.2)

Where the independent variable x represents time, the transform variable \( \xi \) is frequency.
Figure 3.4.1 K-space can control the signal by adjusting the magnitude, frequency and phase. (www.e-mri.org)

2D K-space corresponds to the parallel lines in the matrix (Fig.3.4.2). Gradients along the x (Gx) and y (Gy) directions move the signal position from left to right and up to down. Gy carries fy up one line and Gx carries fx to the right side of the K-space. MR raw data in K-space contains not only the magnitude of the frequency component but phase information as well. For Magnetic Resonance Temperature Imaging (MRTI), phase is more important than the signal amplitude, thus, phase differences induced by temperature changes are calculated from the K-space phase map.

Figure 3.4.2. The image of the Fourier plane is often a magnitude image (gray map), but the amplitude is always associated with a phase information (color map).
3.5 Keyhole Imaging

In real-time temperature monitoring, we are not only interested in temperature spatial resolution, but the temporal resolution as well. In other words, how fast one can measure the temperature is critical. For this reason keyhole imaging is used to increase the temporal resolution while maintaining high temperature image resolution. The center of k-space contributes the most information (contrast and general shape) of reconstructed MR images. Also the center contains low spatial frequency data which has the highest amplitude, giving the greatest changes in gray levels (contrast). High spatial frequency data (at the edge of k-space) have lower amplitude. High spatial frequency contributes to spatial resolution and has minimal effect on contrast or feature shape. The farther from the center of k-space (or the bigger k-space), the higher the spatial resolution will be.

However, MRI is a time consuming process. It requires a much longer time to acquire a high resolution image than a low resolution image. For example, for a high resolution image acquisition of 256 x 256 x 3 with 4 NEX, the total acquisition time is 4min per image which is not acceptable in MRTI guided LITT. In our experiment, the total laser treatment is about 10 minutes. Instead of acquiring a 4minutes of high resolution scans for temperature measurement, we acquire a low resolution image of 256 x 64 x 3 for only 6 sec per image. In the keyhole imaging process, we take these low resolution dynamic images which contain temperature phase information and insert it into the high resolution reference K-space to replace the original one (Fig. 3.5.1). After reconstruction, high temporal resolution temperature image is achieved with relatively high spatial resolution.
Figure 3.5.1 Keyhole imaging replaces the original center K-space with dynamic temperature K-space. The advantage is that the keyhole technique increases temporal resolution without a loss of spatial resolution by limited data acquisition.
CHAPTER FOUR
MULTI-WALLED CARBON NANOTUBES

4.1 Introduction

In 1991, Iijima [13] discovered a new material; Multi-Walled Carbon Nanotubes (MWCNTs), when he was analyzing the carbon soot that resulted from a fullerene synthesis experiment. Single-walled nanotubes (SWCNTs) were also synthesized a few year later [29,30]. Since then, research on these nano-particles has blossomed in many different directions and all over the world. MWCNTs have several concentric cylindrical shells, with an interwall thickness of 3.3 Å. Their diameter is typically in the nanometer range, while their length can vary from 100nm to one millimeters according to different manufacturing conditions [31,32,33]. Fig. 4.1.1 illustrates the structure of MWCNTs. Several thin layers of carbon wall are wrapped together to form the unique finite structure. They have many interesting and unusual mechanical and electrical features. For example, they: have high modulus (resistance towards axial deformation, about 1.2Tpa) [34] ; are good conductors of heat; have the ability to emit electrons or x-rays [35]; serve as
microscopy nano probes [36], secondary batteries and capacitors [37] and enable particle channeling through micro and nanostructures [38-43]. MWCNTs could serve as a gas storage tube or drug delivery tool in nanomedicine by filling a gas or drug in its hollow cylinder structures with the end sealed [44]. After being coated with protein receptors, the tube is also a good candidate for detecting specific targets inside the human body for instance, tumor cells. These characteristics offer considerable opportunities for potential applications in industry and clinic. MWCNTs can absorb and emit light in the near infrared spectrum which makes them potential candidates as the heating generator for thermal therapy [15]. Many studies have reported detailed information about nanotubes’ properties [45,46,47] In this chapter, we are studying Fe containing MWCNTs as superparamagnetic MRI CAs to localize the tumor in vivo. Furthermore, the heating properties of different MWCNTs loaded solutions are tested by applying an NIR laser.

Figure 4.1.1: Schematic of MWCNTs structure. Several graphene layers are rolled up to form the hollow cylinder. Gas, drugs, iron particle can be filled into the middle [11].
4.2 Synthesis and Dispersion

MWCNTs can be synthesized by different techniques: discharge; laser vaporization; solar furnace synthesis and chemical vapor deposition (CVD). In discharge synthesis, an inert gas is filled in the reaction chamber. The position of two graphite electrodes are adjusted until a 50-200 A current occurs. The temperature exceeds 6000°C near the electrodes. As a result, carbon nanotubes are formed in the anode. Laser vaporization is very similar to discharge synthesis, except that a laser is applied instead of high potential and current. In solar furnace synthesis, a mixture of graphite and catalyst (Cobalt, Nickel, and Yttrium) are vaporized by concentrated solar energy with a parabolic-mirror solar furnace. Compared to other synthesis methods, the most controllable method for MWCNTs manufacture is CVD. The technique is suitable for mass production, large area deposition and industrial applications. The MWCNTs used in this project were made using the CVD method at the Nanotech Center, Wake Forest University.

The manufacturing apparatus consisted of a two-stage tubular quartz furnace, the first stage is the preheater and the second is the growth oven (Fig. 4.2.1). Hydrogen was used as a carrier gas with a flow rate of 320 sc/cm. The temperature of the preheater was maintained at 160°C, and the injection feed rate was 5 ml/h. The synthesis temperature ranged from 600°C to 900°C with growth time of approximately 1 hour. After the reaction, the hydrogen gas was switched to argon with the same flow rate so the preheater and the oven would cool to the same temperature. Then nanotubes deposits homogeneously along the length of the heating zone inside the quartz tube [48]. Different Fe containing MWCNTs were produced with different weight concentrations of ferrocene in Xlyene solution as a catalyst (600mg, 400mg, 200mg, 60mg Ferrocene). To synthesize N-doped MWCNTs, a small amount of ferrocene (Fe(C₅H₅)₂), and a carbon-nitrogen source, pyridine (C₅H₅N), were injected into a preheater at a controlled rate of 5 ml/h
using a syringe pump. Thus, N-doped MWCNTs have fewer iron particles compared to Fe containing MWCNTs.

**Figure 4.2.1** chemical vapor deposition (CVD) equipment for the synthesis of Multi-Walled Carbon Nanotubes (Center for Nanotechnology and Molecular Materials).

For biological applications, cytotoxicity is also critical for modification of MWCNTs’ surface. Raw MWCNTs may contain metal catalyst particles and various types of carbonaceous material generated from the CVD production process. In order to use these MWCNTs *in vitro* and *in vivo*, they have to be purified for the application so that iron particles outside the nanotubes are cleaned. For purification, a water solution with 15 mg raw MWCNTs is presonicated in a Bronson 3510 sonicator for 15 minutes [15]. Then the MWCNTs acid solution is ultrasonicated in acid solution mixed sulfuric and nitric acid (90ml sulfuric acid and 30ml nitric acid 3:1 in volume) for 20 hours at room temperature with a cooling system. Then, the suspension is vacuum filtered with 0.2 μm polytetrafluoroethylene (PTFE) Teflon paper dampened with methanol before filtration to ensure the filter was secure. The acid treated MWCNTs solution is washed with 500 ml
DI H₂O. The filter paper is removed, placed in a lab dish and stored in an oven at 60 - 70 °C for 24 hours or longer to allow it to dry. Purified MWCNTs are carefully removed from the filter paper using a blade. The samples are checked using a Philips 400 transmission electron microscope (TEM) (Fig. 4.2.2). Due to the high production temperatures, Ferrocene is dissociated from iron and randomly encapsulated as small iron particles inside the hollow MWCNT channels. A 4 nm by 10 nm iron core is observed in purified MWCNTs with 5-10nm wall thickness. The length varies from 600nm to 1000nm.

![Figure 4.2.2](image)

**Figure 4.2.2** TEM images showing the presence of Fe particles (black dots) in Multi-Walled Carbon Nanotube Scale bar represents 40 nm.

In order to use MWCNTs in biological applications, the nanotubes must be dispersed in water [49, 50]. However, MWCNTs are hydrophobic particles. It is not easy to be dispersed homogeneously in water solutions. Two methods are commonly used to make homogeneous MWCNTs solutions [51-55]. The first method is add water soluble side groups onto chemical functionalized MWCNTs, while the second method is to use surfactants micelles [49, 56-60]. If only the surfactant concentration is above the Critical
Micelle Concentration (CMC), the micellar formation can be maintained. Otherwise, micelles will dissociate into loose multimolecular aggregates. Detailed principles, methods, and applications have been discussed by many groups [49,60-71] Several materials could be used as surfactants, for example, SDS, TritonX 100, Pluronic F68 (Poloxamer 188), Pluronic F108 (Poloxamer 338) and Pluronic F127. SDS and TritonX are known to be toxic. Pluronic F127 is a Food and Drug Administration (FDA) approved surfactant [64]. Also it is well tolerated by cell cultures [63]. We think it is the best candidate to disperse MWCNTs for \textit{in vivo} or \textit{in vitro} study. First 1wt\% Pluronic F127 is dissolved in the water. MWCNTs are added at a certain concentration. Then, the solution is sonicated for about 15 minutes until the MWCNTs are dispersed evenly in the water. Pluronics are PEO-PPO-PEO triblock copolymers long chains. It extends into water and prevent MWCNTs from reaggregation and deposition. Moreover it has been reported that 30wt\% can form a gel at physiological temperature [67].

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure423.png}
\caption{MWCNTs particles (right) and homogenously dispersed MWCNTs solution using Pluronic F127(left).}
\end{figure}
4.3 MWCNTs as MR contrast agents

The purpose of using an MRI CA is to modify local T$_1$ or T$_2$ properties of the hydrogen nuclei located in their vicinity, resulting in higher contrast for the region of interest compared to the MR image contrast without adding CA. Magnetic relaxation properties vary as the square of the magnetic dipole moment which scales with the number of unpaired electrons in the outer shell of the atom. Thus, the number of outer shell unpaired electrons determines the relaxation effects.

There are two main classes of contrast agents: The first class is Paramagnetic: Gadolinium chelates, used for T$_1$ weighted sequences. The second class is ferromagnetic, typically ferrite particles, used for T$_2$ weighted sequences. The basic physics of MRI contrast agents has been reviewed [72,73]. Paramagnetic substances' magnetic properties will appear when there is an external magnetic field. If the field is removed, atomic dipoles will return to random orientation. As a result, the coherent effect will be lost. Ferromagnetic substances remain polarized after the externally magnetic field is removed. There are only a few ferromagnetic substances (iron, cobalt and nickel). The combination of paramagnetic and ferromagnetic substance is called superparamagnetic. They have extremely large magnetic moments, which are acquired by these molecules in the presence of an external magnetic field. This makes superparamagnetic particles very efficient for MRI CA purposes. The efficiency of change in the relaxation properties upon CA injection in tissue is measured by defining the relaxivity R$_1$ and R$_2$ using equation:

\[
\left( \frac{1}{T_i} \right)_{\text{total}} = \left( \frac{1}{T_i} \right)_0 + M \cdot R_i.
\]  

(1/Ti)$_{\text{total}}$ is the relaxation rate in the presence of a contrast agent and (1/Ti)$_0$ is the relaxation rate of a water molecule. M is the effective concentration of paramagnetic or superparamagnetic metal ions, (for example, Fe: 0.5mM). i = 1 holds for longitudinal relaxation T$_1$ and i = 2 holds for transversal relaxation T$_2$. The higher R$_1$ or R$_2$, the more...
efficient is a contrast agent or the higher concentration of metal ion. Also iron core inside MWCNTs will change the proton nuclear spin relaxation of water molecules in the vicinity. In a preliminary in vivo experiment, we injected 200ug of 600mg Fe MWCNTs into tumor bearing mice. The results show the successful negative contrast inside the tumor in both 1.5T GE scanner and 7T Bruker animal scanner (Fig. 4.3.1 and Fig.4.3.2). Both images clearly show the position where these Fe containing MWCNTs were injected. However, whether the MWCNTs are stable (relaxation property) after laser irradiation and where MWCNTs will migrate after laser treatment is still not clear. As a result, the stability of Fe containing MWCNTs quantitively was investigated by measuring T2 relaxation properties and tracking the MWCNTs position in vivo before and after the treatment.

![Figure 4.3.1](image-url)

**Figure 4.3.1** MR structure coronal images in 1.5 T: before (a) and after (b) injection of 600mg ferrocene MWCNTs solution. The dark area shows the MWCNTs inside the tumor. (Dr. Michael Schmid unpublished)
Figure 4.3.2 MR structure axial images 7T MR scanner before (a) and after (b) injection of 600mg ferrocene MWCNTs solution. The dark areas show the MWCNTs inside the tumor.

Methods

Three kinds of Fe containing MWCNTs (600mg, 200mg, 60mg ferrocene) were suspended in Pluronic F127 1% wt DI water to test their T2 relaxation properties. The dilution series (from 0.5mg/ml to 0.03mg/ml) were aligned in a matrix phantom and scanned in the 1.5 T GE scanner. Fig. 4.3.3 is the MR image coronal slice of the phantom. Transverse (T2) relaxation properties of the MWCNTs solution were measured from FGR pulse sequence with parameters: TR=4.8ms, TE= 1.3 ms, the flip angle= 30° the number of excitations per acquisition (NEX) = 1. matrix size is 256 by 128. Data fit the signal exponential decay due to the transverse relaxation properties described in Chapter Two. The 1/T2 (R2) value indicates the iron concentration of different Fe containing MWCNTs solutions (Fig. 4.3.4). The derived 1/T2 shows that 600mg ferrocene MWCNTs have the highest iron concentration per mass among all the MWCNTs. Iron percent weight of MWCNTs was measured independently using Inductively Coupled Plasma (ICP) methods (Table 4.3.1) (courtesy of laboratory of EL Huffman, Ph.D.). Then, the
concentration of MWCNTs in solution (mM) is derived from percent weight per ml (mg/ml) according to the ICP results.

**Table 4.3.1 iron concentration (percent weight) measured with Inductively Coupled Plasma (ICP)**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Percent weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>600mg Fe MWCNTs</td>
<td>2.92</td>
</tr>
<tr>
<td>200mg Fe MWCNTs</td>
<td>1.71</td>
</tr>
<tr>
<td>60mg Fe MWCNTs</td>
<td>1.26</td>
</tr>
</tbody>
</table>

![Figure 4.3.3 MRI coronal structure image of MWCNTs dilutions. Line 1 to 3 is 60mg, 200mg, 600mg Ferrocene MWCNTs with dilution series from 1-9 (0.5 to 0.03mg/ml). Water reference solution is use as an asymmetric control object.](image)

In order to fully understand their stability *in vivo*, we began a non-invasive follow-up study to evaluate the long term biodistribution of the Fe containing MWCNTs in tumor before and after the laser irradiation. Due to their unique relaxation properties, the MWCNTs injected tumor's T2 value change could be measured from T2 weighted MR images. The breast tumor of the mouse was injected 100ug of 600mg Ferrocene MWCNTs at concentration of 2mg/ml (Fig. 4.3.4). T2 values were obtained on a pixel by pixel basis from the Multi-Slice Multi Echo pulse sequence image (MSME-T2-map) with Matrix: 128 x 128 Pixel size: 0.23x0.23mm FOV: 3x3 cm TE= 10, 20, 30,… to 160ms (10ms increment) TR =2000ms. The signal intensity is fitted as an exponential decay from the multiecho image set (Fig. 4.3.5). The mouse was scanned before the MWCNTs
injection, after the MWCNTs injection, after the laser treatment, 24 hours post-treatment and one week post-treatment in order to study the long-term stability of the MWCNTs biodistribution in tumor.

![Image](image.png)

**Figure 4.3.4** a breast tumor bearing nude mouse was injected with 100ug of 600mg Ferrocene MWCNTs solution with concentration 2mg/ml.
A series of T2 weighted image at different echo times TE increments is 10ms (10ms, 20ms...160ms) by MSME pulse sequence. The intensity in a pixel of the T2 weighted images decays exponentially with a time constant T2. T2 value is then calculated from these series pixels.

**Results**

A remarkable result shows that Fe containing MWCNTs manufactured using 600mg Ferrocene’s has the highest R2 (549 mM⁻¹s⁻¹) which is about five times higher than the clinical CA, Feridex (107 mM⁻¹s⁻¹) (Table 4.3.2). This exciting result indicates that the MWCNTs could potentially be used as an effective MR contrast agent.
Figure 4.3.6 MR measurement of 1/T2 as a function of concentration (mg/ml)

Table 4.3.2 T2 relativities R2 (1/T2) measurements. Compare to the commercial MR contrast agents Feridex, R2 is unusually higher than commercial MR contrast agents Feridex and Resovist. This opens a new possible application in T2-weighted MR contrast agents.

<table>
<thead>
<tr>
<th>Sample</th>
<th>R2 mM·s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>600mg Fe MWCNTs</td>
<td>549</td>
</tr>
<tr>
<td>200mg Fe MWCNTs</td>
<td>481</td>
</tr>
<tr>
<td>60mg Fe MWCNTs</td>
<td>483</td>
</tr>
<tr>
<td>Feridex IV</td>
<td>107</td>
</tr>
<tr>
<td>Resovist</td>
<td>190</td>
</tr>
</tbody>
</table>

An in vivo experiment (Figure 4.3.5) shows ventilation images at different imaging time points (before and after MWCNTs injection, after laser treatment, 24hours post-treatment and 7 days post-treatment) with corresponding T2 value of the Region of Interest (ROI). The T2 relaxation inside ROI (yellow circle in Fig. 4.3.5) was 87.0ms ±/−
5.0ms before injection which reduced to 24.4ms +/-2.4ms the after injection. The Fe containing MWCNTs remained stable in the tumor after 3W/cm² 30seconds NIR irradiation (24.1ms +/-3.6ms). We observed that T2 relaxation in the ROI dropped slightly after 24 hours post-treatment (19.6ms +/-2.5ms) and 7 days post-treatment (17.5ms +/-4.1ms). This T2 change might be due to the tumor tissue damage from the high temperature after the laser treatment. In this study, iron core inside the MWCNTs were large enough to induce a significant drop in T2 value. The images show the exact position where the 600mg Ferrocene MWCNT was injected before the laser treatment as well as one week post-treatment. This significant finding indicates that the Fe containing MWCNTs can be used as long term negative-MRI CA (dark enhancement contrast) even after the LITT. Thus, the MWCNTs injected tumor can be located and retreated without additional MWCNTs injection.

Discussion

MWCNTs external surfaces can be used as a scaffold for attaching a wide variety of agents such as antibodies or peptides, biocompatible coatings and water solubilizing groups like Pluronic F127. The ultimate goal of an MRI CA is to accumulate in the specific organ or target after intravenous injection instead of direct injection into the designated target. The exceptionally large R2 relaxivities of Fe containing MWCNTs could provide sufficient signal-to-noise ratio (SNR) (Fig.4.3.1) to image targeted tumor. Recently, SWCNTs have been shown to translocate into the interior of cells with minimal cytotoxicity [105-108]. Thus CAs made of these nano materials could also accumulate inside the target tumor or cells to further boost the concentrations of the nano particles which boost MRI contrast. Therefore, specifically coated or chemical modified MWCNTs could be used for early detection of cancer cells.

The Fe containing MWCNTs also show the potential of being the MRI image-guided therapeutic agent that can be used for targeted area in thermal therapy by
locating the target and then enabling its heating. In next section, the heating properties of these noval nano particles are investigated.

**Figure 4.3.7** T2 relaxation measurement from MSME pulse sequence before MWCNTs injection, after MWCNTs injection, after laser treatment, 24 hours post-treatment and 7 days post-treatment.
**MWCNTs solution stability**

As a potential clinical MR CA as well as a remarkable NIR laser absorber and heat generator, the stability of MWCNT solutions need to be well understood. Although Pluronic F127 is well known for dispersing the MWCNTs, we have found out that the MWCNTs re-aggregated at the bottom of the vial after 2 months (Fig. 4.3.8). However, there is no clear definition for MWCNTs suspension stability and no quantitatively study about MWCNTs deposition rates. In this section, we will discuss a method to measure the stability of MWCNT solutions using MRI. As reviewed, transverse relaxation properties (\(1/T_2\)) are proportional to the iron concentration. Assuming the iron core inside Fe containing MWCNTs will not leak out in the solution and that every nanotube has about the same amount of iron core. Then the concentration of MWCNTs can be indirectly measured according to the relaxation properties of the solutions.

*Figure 4.3.8* a) a well dispersed MWCNTs solution after 15 min sonication. b) a vial of MWCNTs solution after two month. The MWCNTs reaggregated at the bottom of the vial.
To test the stability of the MWCNTs solution, 60 mg Ferrocene MWCNTs dispersed in 1% wt Pluronic F127 DI water and is sonicated for 15min until MWCNTs is well dispersed in the water. The vial of MWCNTs is set up in the 1.5T GE MR unit for two days while measuring T₂ relaxation time continuously (about 20min per scan). Data are normalized according to the initial iron concentration relaxation properties. Figure 4.3.9 illustrates the relative iron concentration as a function of time throughout 2 days scan. At the fifth day, we scan again before and after shaking to check the concentration change. The interesting results show that the “stable” MWCNTs is not stable at all. The two days continuous scans show the concentration of the solution drop from 100% to 82%.

After about 5 days, the concentration drops to 48% based on the measured relaxation time. After shaking the solution for a short period, the concentration returned to the original one. (Table 4.3.3) These experiments indicate that the Pluronic F127 dispersed MWCNTs solution has a slow re-aggregation and deposition rate. We suggested shaking the solution every time before using in order to keep the same concentration.

![Figure 4.3.9](image)

**Figure 4.3.9** Relative Fe concentration in the solution decrease as a function of time which indicates the deposition rate of a well suspend MWCNTs solution.
Table 4.3. 1/T2 value and relative iron concentration before and after shaking

<table>
<thead>
<tr>
<th>Relaxation properties</th>
<th>Original solution</th>
<th>After 5 Days</th>
<th>After shaking</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/T2</td>
<td>129 ms⁻¹</td>
<td>62 ms⁻¹</td>
<td>128 ms⁻¹</td>
</tr>
<tr>
<td>Relative iron concentration</td>
<td>100%</td>
<td>48%</td>
<td>93%</td>
</tr>
</tbody>
</table>

4.4 MWCNTs as NIR absorbers and heat generators

In this section, MWCNTs solutions’ heating capabilities are tested in the presence of NIR laser (1064nm) to exploit the feasibility of using MWCNTs as an effective NIR absorber for LITT in vivo. MWCNTs’ electromagnetic wave absorbance is most efficient when the antenna length is a multiple of the half of wavelength of the radiation [74,75], according to Jackson’s classic antenna theory [14]. Therefore, NIR light with wavelength 1064nm can strongly couple with our 1000nm long MWCNTs. Besides the antenna size, the efficiency of absorption also depends on the antenna shape. Particle without a linear rod shape has lower absorption efficiency. Metallicity or in other words the electronic band structure also determines the absorption efficiency. MWCNTs consist of several coaxially aligned SWCNTs, which are metallic nanotubes. This makes MWCNTs more efficient than SWCNTs in NIR absorption and heat generation (Fig. 4.4.1) [16]. MWCNTs and SWCNTs were suspended in 1% (wt/wt) Pluronic F127 saline solution. The temperature is measured by a thermal couple after exposure to a NIR laser (1064nm wavelength) 3W/cm² 30 seconds. 0.1 mg/ml solution of MWCNTs increased from 23°C to 51°C +/- 0.7°C compared to the SWCNTs from 23°C to 27°C +/- 0.3°C. The graph shows the MWCNTs have the stronger ability to induce higher temperature at a low concentrations and short laser exposure time. It suggests that MWCNTs may be useful as photothermal mediators.
MWCNTs absorb photons and convert their energy into heat. The efficiency of electromagnetic light waves coupling to a particle can be described as the probability of a photon being absorbed and re-emitted by an individual atom. With each atomic absorbance and re-emission, the photon loses energy which is converted into phonons and therefore into heat. For a metallic system with a metallic density of electronic states, this probability goes as the oscillator strength of the particle. This oscillator strength is also called the antenna-power. According to Beers law, the laser intensity decreases exponentially as a function of penetration depth due to the attenuation. For biological targets at depth, only a small percentage of laser energy hit is able to penetrate the tissue and reach the target area. However, by using MWCNTs as a high efficiency NIR absorber and heat generator, even a small fraction of laser energy delivered can produce enough heating to destroy the tumor underneath the skin.

Here, we are studying the heating properties of different MWCNTs at presence of NIR Nd:YAG (neodymium-doped yttrium aluminum garnet) laser with wavelength

**Figure 4.4.1** MWCNTs produce a greater temperature increase than SWCNTs in response to NIR illumination. MWCNT and SWCNT were suspended at a range of final concentrations in saline containing 1% (wt/wt) Pluronic F127 and illuminated at 3 W/cm$^2$ for 30 sec with a 1064-nm NIR laser. Temperature was measured by thermocouple. Shown are mean and standard deviations of triplicate measurements. (Inserted fig) Detail on the dilution range from 1 to 100 mg/ml. [16]
1064nm. These experiments were performed with 4 groups of different MWCNTs (600mg, 400mg, 200mg, 60mg Fe and N-Doped MWCNTs) with the same concentration 0.1mg/ml. MWCNTs were dispersed in media and irradiated for 3W/cm² for 30, 60, 90 seconds. As shown in Figure 4.4.2, an increase in temperature was attained when the nanotubes were irradiated by NIR Nd:YAG laser. Five groups of different kinds of MWCNTs have almost the same high efficiency of generating the heating. Compared to the control media without MWCNTs, the temperature increase is significant. As a result, all the MWCNTs have super heat generation abilities and could be used in the following in vivo and in vitro (phantom) experiments.

**Figure 4.4.2.** Different kinds of MWCNTs have the same heating properties. Four groups of MWCNTs were irradiated using 1064nm NIR Nd:YAG (neodymium-doped yttrium aluminum garnet) laser 3W/cm² with 30, 60, 90sec. The result show the four MWCNTs increased about the same temperature at presence of different laser dose. Initial temperature is 21 ºC.
5.1 Introduction

In general, there are two kinds of thermal therapy based on the temperature levels and heating time used. The first is called hyperthermia, where temperature is elevated and maintained in the range of 43-45 °C for about half hour to kill cancer [18]. The second kind is called thermal ablation. Compared to the hyperthermia, its temperature range is 50-80 °C or above for a short time. This high temperature will result in tissue coagulations and necrosis [19]. Due to the dynamics of tissue heating properties and blood flow during thermal treatment [76-79], the shortcoming of most ablative approaches is the inability to monitor the 3D spatial temperature distribution non-invasively during the therapy. To overcome the thermal therapy drawbacks, recently, PRF based MRTI has been developed to monitor the real-time 3D temperature distributions during the treatment. Furthermore, as shown in this work, MWCNTs have
been found as an effective NIR laser absorber and heat generator for an approach that could kill the cancer cells in vitro [15]. This new thermal therapy which combines two new techniques becomes very attractive for two reasons. First, the local thermal damage can be controlled by the injection of MWCNTs. Second, the high spatial and temporal resolution MRTI can be used as a quick feedback during the treatment, which provides protection for the healthy tissue around the lesion. Ideally, surrounding healthy tissue could be totally avoided while the tumor labeled by MWCNTs is ablated. The goal of this project is study the feasibility of combining MWCNTs and MRTI in LITT.

In this chapter, fiber optic and external MR compatible laser systems are described for their use with a 7T MR animal scanner. Three different gel phantoms models (alginate-only, MWCNTs-instilled, and a subcutaneous tumor phantom) were made for MRTI-guided-LITT experiments to simulate the heating result in vivo. Then, kidney tumor bearing mice were treated non-invasively using a low power external NIR laser beam while temperature changes were monitored using 7T MR animal scanner. The tumor volumes are tracked post-treatment to evaluate the effect of this new thermal therapy.

5.2 Gel phantom manufacture

The chemical compound sodium alginate (NaC₆H₇O₆) is a sodium salt of alginic acid. Extracted from the cell walls of brown algae, this chemical compound is used by the foods industry to increase viscosity and as an emulsifier. Since it is non-toxic, tissue equivalent for radiological and MR imaging and easy to make, it is also used in many biological experiments. In this project, we have utilized these sodium alginate based gel phantoms to simulate healthy tissue or tumor with different shapes (Fig. 5.2.1).

1) To make an alginate-gel phantom: 3g Sodium alginate powder is dissolved in the 100ml deionized (DI) water to make a bottle of viscosity alginate solution (3%wt).
Pour the alginate solution into 6-well plate model and cover the viscosity solution with a 0.2μm filter to prevent the leakage. After filling a 6-well plate with alginate solutions, put the plate into 1%wt Calcium Chlorite (CaCl₂) solution and let filter covered sodium alginate solution react with calcium chlorite completely. The sodium alginate is a viscous solution. The alginate is a long molecule that has lots of negative charges. When the sodium alginate is added to the calcium chloride solution the calcium ions displace the sodium ions and form a gel. The gel is formed because the calcium ion is divalent and cross links the long chains of alginate. During this process water is squeezed out from between the alginate molecules and gel is formed after three days reaction.

2) **To make a MWCNTs distilled gel phantom:** MWCNTs water suspensions are mixed into alginate solution. Afterwards, the mixture of gel solution is shaken for 5 minutes until MWCNTs are homogenously distributed and then the mixture is poured into the 6-well or 48 well plates according to the different requirement of size. The mixed solution is then reacted with Calcium Chlorite.

3) **To make a subcutaneous tumor phantom:** After the solid MWCNTs phantom is formed, drop one small MWCNTs phantom into one well of a 6-well plate and then pour alginate solution to cover the whole MWCNTs phantom. Then the 6-well plate is soaked in NaCl₂ solution.

**Figure 5.2.1** (a) a alginate-only gel phantom simulating the normal tissue without MWCNTs. 4 temperature probes are inserted into the middle to measure the temperature. b) a thin layer of MWCNTs distilled phantom to simulate the tissue with MWCNTs. Fiber laser is applied to test its heating properties. c) subcutaneous phantom which simulates the situation where tumor with MWCNTs is underneath the skin and surrounded by health tissue without MWCNTs.
5.3 MR compatible Laser system design

There involves safety and compatibility issues to move the laser into the MRI scanner room because most of the high power laser system is not MR compatible. To perform the experiment in the MR scanner, we designed our own MR compatible fiber laser and external laser system, which could extend the laser beam into the 7T MR animal scanner to enable simultaneous laser heating and MRTI. Based on tumor location, we can have the choice to treat tumor minimally-invasively for tumor deep inside the body with a small optic fiber cable or non-invasively for superficial tumor by using external laser.

In the small optic fiber laser setup, the original laser beam (1cm in diameter) is focused into a spot (1mm in diameter) so that a fiber optic cable can transfer the NIR laser energy into the MR scanner room. A mount with a microscope lens was installed in front of the portable laser system and the NIR laser beam was focused to the tip of the fiber laser cable through the microscope lens. (Fig. 5.3.1). A six-meter long optic fiber plastic cable transfers the laser light to the target inside the 7T MR scanner. The efficiency of energy transfer is about 6%-12% depending on the alignment of focusing spot and optic fiber cable tip.
Due to the limited space in the 7T animal MR scanner (only 15cm diameter), colleagues C Rylander, Ph.D. and graduate student Chris Drew designed a compact external laser holder for our noninvasive MRTI guided LITT mice study. The device has a plastic holder and tube that can hold the animal and transfer the laser beam to a desired target location. A diffraction collimator is attached to end of fiber optic cable to expand the fiber laser beam back to 1cm diameter wide. The special NIR minor has 98% reflection efficiency and bends the laser 90 degrees so that the external laser beam can treat the superficial tumor from the top. A 35mm surface RF coil is attached to the head of the laser holder for simultaneous 7T MR imaging. The advantage of the surface coil compared to an axially aligned coil that holds the entire animal is that the surface coil provides a much higher SNR at the superficial region of interest (ROI) and gives more
space for animal setup in the small 7T animal MR unit. Figure 5.3.1 illustrates the MR compatible external laser beam setup for the 7T MR animal scanner.

![MR compatible external laser beam design](image)

**Figure 5.3.2** MR compatible external laser beam design for 7T MR Bruke animal Scanner

### 5.4 Phantom Model Experiment

It is important to know the heating properties of MWCNTs in a tissue equivalent phantom before *in vivo* experiments proceed. Thus, our new MRTI guided LITT with MWCNTs experiments were first performed in three tissue equivalent gel phantoms (Fig.5.4.1 simulating healthy tissue, MWCNTs injected into tumor, and the subcutaneous tumor, with composition as described in the previous section).

**Methods**

The first minimally-invasive MRTI guided LITT experiment used fiber optic laser delivery to test the different heating results in the MWCNTs instilled phantom and the alginate-only gel phantom. A thin fiber optic laser with diameter 0.6mm was inserted into
the middle of the alginate-only gel and MWCNTs instilled phantoms. Fig. 5.4.1 shows the phantoms and fiber optic cable positions. Both phantoms were irradiated for one minute using NIR laser (1064nm) with 0.1W.

![Figure 5.4.1](image1.png)

**Figure 5.4.1** illustrates the MWCNTs instilled phantom relative location in the alginate-only phantom and the laser beam direction

In the second non-invasive MRTI-guided-LITT experiment, the external laser setup was used to test non-invasive thermal therapy towards the subcutaneous tumor surrounded by healthy tissues (Fig. 5.4.2). The MWCNTs instilled phantom was implanted 4mm underneath the gel phantom, simulating a subcutaneous tumor. External NIR laser with diameter 10mm and power 1.5W were turned on for 30seconds.

![Figure 5.4.2](image2.png)

**Figure 5.4.2** illustrates the MWCNTs instilled phantom relative location in the alginate-only phantom and the laser beam direction
Instead of using thermocouple temperature measurements (described in Chapter 4), MRTI is applied for non-invasive 3D temperature measurement of phantom during the heating. A FLASH gradient echo sequence was used to measure the phase differences for temperature monitoring. A high resolution reference image for keyhole imaging was first acquired with parameters: Matrix Size= 256 x 256; FOV= 4cm x 4cm x 4cm; FA=15 degree, TE=5.1ms TR=50ms. This image was followed by fast low resolution temperature runs with parameters: resolution 256 by 64; FOV 4cm x 4cm x 4cm; FA=15 degree, TE=5.1ms 80 frames, temporal resolution 9s (9s each temperature measurement).

**Results**

With fiber optic laser heating (ø 0.6mm, 1 min @ 0.1W) inserted in the middle of the phantom and irradiation from right to left, the MWCNTs instilled phantom heats much faster and reached a much higher maximum temperature (from 20°C to 47°C; Δt=+27°C), compared to the alginate-only phantom (from 20°C to 25°C; Δt=+5°C Fig. 5.4.3).

![Figure 5.4.3.](image)

**Figure 5.4.3.** MWCNTs-instilled alginate gel phantom (a, b) and alginate-only gel phantom (c, d) show that MWNTs generate more heat for the same laser power and irradiation time (fiber laser 1W/cm² 1min exposures). (a,c) MR images of both phantoms. (b, d) The MR temperature images are taken at the maximum temperature during the Laser radiation. (e) Temperature in different phantom near fiber laser during the heating process.
With non-invasive laser heating (ø 10mm, 0.5 min @ 1.8W), the implanted region of the MWCNTs-implanted phantom showed significantly elevated temperatures compared to the nearby alginate-only medium (Δt=+15°C). (Fig. 5.4.4)

![Image of laser heating experiment](image)

**Figure 5.4.4** External Laser beam (1.8w/cm²) heating of a MWNT gel phantom implanted in the center of the alginate-only gel phantom, simulating a sub-cutaneous tumor. (a) implanted phantom MR image. (b) MR temperature image taken at the maximum temperature during procedure. (c) Temperature curve along the laser path in two pixels.

**Conclusion**

The successful phantom experiment established a new platform for study of laser thermal therapy by monitoring the 3D temperature distribution using MRTI. These phantom models and their heating results measured by MRTI match the previous liquid solution heating experiments using a thermocouple. The MWCNTs can absorb NIR laser energy and energy heating in both solution and tissue-equivalent gel phantoms. The second non-invasive LITT experiment using the subcutaneous MWCNTs tumor indicates that even if the tumor is underneath the skin, it can still be heated by the small fraction of laser energy that penetrates the skin. The 15 degree difference between the temperature of thin layer of gel and that of the MWCNTs instilled phantom in the middle also indicates that tumor under the skin could be treated non-invasively while the healthy tissue is protected from thermal injury. **The in vivo** tumor model experiment is built on this phantom model.
5.5 Animal Model Experiment

Cell culture and tumor preparation

Cell culture was first devised at the beginning of this century by Harrison in 1907 and Carrel in 1912 [80]. It is a technique for growing cells outside the human body under laboratory conditions. RENCA kidney cancer cells are used in animal models for the study of cancer. Kidney cancer is more common in people between the ages of 50 and 70, and affects men almost twice as often as women. Smokers also develop kidney cancer about twice as often as non-smokers. Surgery is currently the main treatment for kidney cancer. The thermal sensitivity of RENCA cancer cells is similar to the other cancer cells such as MCF-7 breast cancer, PC-3 prostate cancer, Caki-1 kidney cancer, and HeLa cervical cancer cells. All of these cancer cells can be killed by NIR laser in vitro [16]. RENCA kidney cancer cells were chosen for the in vivo study of MRTI-LITT for and implanted flank tumor in mice. These cells were cultured under standard conditions and 2mm³ RENCA tumor fragments were implanted into the right flanks of 12 mice. The tumors reached 5.5mm x 5.5mm x 0.5mm in greatest dimension (about a week) before undergoing MRTI-LITT. These mice were separated randomly into 3 groups (MWCNTs, Saline, Control n=4 per group). There was no statistical difference among tumor sizes or animal weights for the groups. Twenty-four hours before the treatment, the MWCNTs group was injected intratumorally with 100 μg of MWCNTs. The saline group was injected saline solutions instead of MWCNTs as a comparison.

Methods

In MRTI-guided-MWCNTs-LITT in vivo experiments, mice were anesthetized with isoflurane. Fig. 5.5.1 illustrates the in vivo experiment setup. Core body temperature was maintained at 35°C during the treatment with a small animal MRI compatible computer controlled heating system, (Small Animal Instruments (SAI)), which blows hot air through the scanner, A 35-mm transmit/receive surface coil (Doty Scientific) was placed around
the tumor and an MR-compatible external laser beam collimator assembly was placed over the tumor such that the laser incidence on the tumor was orthogonal (Fig. 5.5.1). Five-millimeter tubes filled with 0.1 mM solution of Magnevist, a gadolinium-based contrast agent, (Bayer HealthCare Pharmaceuticals) were placed around the laser aperture. The tubes were used as fiducial markers to determine the position of the laser with respect to the tumor on the MRI images. A high-resolution proton density weighted 3DMR sagittal image was acquired with a spoiled gradient-echo FLASH pulse sequence across the mouse tumor with the following parameters: TE = 3 ms, TR = 7.6 ms, FA = 5°, FOV = 3 cm x 3 cm x 2 cm and matrix size = 120 x 120 x 80, pixel size = 0.25 mm x 0.25 mm x 0.25 mm. After the structural images were acquired, a time series of low-resolution temperature sensitive MR images were acquired with identical parameters as the previous scan except for the following parameters: Matrix size = 120 x 32 x 20, pixel size = 0.25 mm x 0.9375 mm x 1 mm and temporal resolution of 4.2 seconds. The low resolution image was acquired continuously for 13 min during laser treatment producing 186 volumes of data throughout the treatment. During the low resolution scan the 1064-nm NIR fiber laser (beam diameter: 1 cm, power density: 3 W/cm²) was turned on the 3rd min for an interval of 30 seconds. MRI image acquisition continued for another 9 min and 30 seconds after treatment to allow the temperature of the tumor to return to back to pretreatment levels. All image reconstruction, temperature map calculations, and analyses were done by custom software written in Matlab (Mathworks). The temperature change between images were calculated from their phase difference and scaled appropriately to convert to relative temperature maps. A cumulative sum of these temperature differences revealed the relative temperature changes of the tumor during laser treatment. A base temperature increment of 26°C (mouse body surface temperature, measured prior to laser delivery) was added to every pixel in the relative temperature map to produce absolute temperature maps.
Figure 5.5.1 MRTI guided LITT setup in 7T animal scanner with external laser beam irradiation \textit{in vivo} experiment.

\textbf{Results}

The MR intratumoral temperature distribution shows the maximum temperature reached 75°C in MWCNTs injected tumor and saline injected only reached 45°C (Fig.5.5.2). Tumor growth was monitored for two weeks until the tumor volume exceed 1200mm$^3$. Control tumors (untreated control, saline control) grew rapidly and uniformly. These two groups’ tumor volumes exceed 1200mm$^3$ in 14 days post-treatment. In contrast, the MWCNTs group tumor volume decreased after MRTI-guided-MWNCTs-LITT with single fraction laser treatment (3W/cm$^2$, 30s) (Fig. 5.5.2.b). The remarkable tumor growth inhibition results shows 80% of mice treated with the combination of MWCNTs and MRTI-guided-LITT were tumor-free after 2 weeks of study (Fig.5.5.2.b). There was no permanent skin injury due to heat injury and any injuries healed over time (Fig. 5.5.3.a) The study ended after two weeks when all saline groups and control group tumor exceed the critical volume.
Figure 5.5.2. MRTI-measured intratumoral temperature distribution during thermal therapy. (a, b, c,d) RENCA kidney tumor bearing mice received MRTI-guided thermal therapy using MWCNTs. (e, f) RENCA tumor bearing mice only received laser radiation without MWCNTs injection. (b, d) Significant heating is generated in the MWCNTs tumor compared to the tumor without MWCNTs injection, as shown in the temperature map on the right. (g) Temperature versus time plot detailing mean temperature in the center of the tumors following 30s of NIR laser exposure [16].
Figure 5.5.3. MRTI guided thermal therapy using MWNT reduces tumor volume. (a) Mice from three groups: Control, Saline, MWCNTs-injected. The mice who received MRTI guided LITT using MWCNT were effectively under control while others (control and saline groups) tumors are still growing. The study ends after 2 weeks' tracking. (b) tumor volume before and after the MRTI-Guided-MWNTs-LITT [16].
Conclusion

In this chapter, a new method is described for thermal therapy of tumors using MWCNTs and a low-power NIR laser heating. After 3W/cm² 30 second irradiation, an 80% tumor regression rate without recurrences was observed after two weeks of treatment for the group of animals with tumors injected with MWCNTs. As a comparison, the flank tumors in both control and saline groups continued to grow. MRTI results showed that the MWCNTs injected tumors reached 76 °C after 30second irradiation while saline tumors without MWCNTs only reached 46 °C (Fig. 5.5.2). This study indicates the location of MWCNTs must be carefully controlled to avoid injury to healthy tissues nearby. Such control is achieved by direct injection of MWCNTs solution into the tumor and by using reduced laser power. MWCNTs delivered by this method appeared well-distributed (Fig. 5.5.4) [16]. These data suggest that the new therapy of combining MWCNTs and NIR laser may potentially be used in such superficial tumors in clinic. The MR compatible MWCNTs and fiber optic laser setup enable this simple but precise heat delivery to be proved for in vitro and in vivo setting. The technique limits the heat injury to the adjacent healthy tissues while causing complete ablation of the tumor (for a simple flank tumor model). This new therapy, MRTI-guided-LITT-MWCNTs, becomes a multifunctional platform for further investigation of this method of tumor treatment.

Figure 5.5.4. MWCNTs in mouse tumor tissue. MWCNTs were injected directly into a s.c. kidney tumor grown in the flank of a nude mouse. Several weeks later, the tumor was laser treated, removed, preserved in paraformaldehyde, and embedded in paraffin. Sections were prepared for light and polarized light microscopy. Additionally, some tumor tissue was fixed with glutaraldehyde, embedded in epoxy resin, thinly sectioned by using a diamond blade, and the sections transferred to formvar-coated copper grids for TEM analysis. Operating voltage was 80 keV. (A) Polarized light micrograph; nanotubes are visible as areas of birefringence throughout the tissue. (B and C) TEM images of the same tumor. [16]
CHAPTER SIX

DISSCUSSION AND CONCLUSION

6.1 Discussion

MRTI experiments

The MRTI guided LITT experiments performed in this study demonstrate that the capabilities for monitoring 3D high spatial and temporal resolution temperature distributions in vivo using a 7T MR animal scanner during laser thermal therapy. However, several issues could affect the temperature imaging qualities. The sensitive temperature MR temperature gradient echo sequence is also sensitive to motion artifacts. As a result, motion artifacts create ghost images along the phase encoding directions which cause inaccuracies in temperature data analysis. Such artifacts happen when an animal is not positioned properly or due to respiratory motion.

Besides the motion artifacts of in vivo experiments, fat chemical shift artifacts affect the MRTI accuracy in vivo. The lipid molecule screens the water proton resonance frequency (Larmor frequency) from shifting corresponding to the different external
magnetic field strength. Since acquisition of the raw-data on K-space is based on Lamor equation (Equ.2.2.2), inaccurate Larmor frequency shift due to the fat signal leads to image shifting along the frequency encoding direction on MRI. Moreover, the MRTI is based on temperature sensitive water proton resonance frequency. Because of the lipid screen effect, water protons surrounded by fat are not affected by temperature as much as should be expected. Consequently, fat MRTI measurement remains the same although the actual temperature is changing.

These two artifacts are the major difficulties of applying MRTI guided LITT in the human body since the human body contains fat component as well as unavoidable respiratory and physiological motions. As a result, the best anatomical site for MRTI guided LITT applications is the brain and this new therapy is using now in clinic trials. In May 2009, MD Anderson Medical Center verified the MRTI guided LITT for Metastatic Brain Tumor. Cleveland Clinic also reported their first treatment of brain tumor patient in February 2009 on ABC news.

**MWCNTs Toxicity**

Nanotubes have unique electrical, mechanical, and thermal properties, which give them wide and potential applications in the electronics, computer, aerospace, nanomedicine and other fields. Everyone is curious whether these carbon based materials can be used in the human body. Their major manufacturing processes, described in Chapter 4, are discharge, laser ablation and chemical vapor deposition (CVD). These synthesis processes involve catalytic metals e.g. iron and there are a lot debates about the MWCNT induced toxicity for humans [89]. Toxicity may depend on the nanotube type, size, shape, and surface characteristics. In several rodent studies test dusts were administered intratracheally or intrapharyngeally to assess the pulmonary toxicity of manufactured CNTs. The results of these rodent studies showed that these CNTs were capable of inducing inflammation in the lungs. [90]
In the experiments, reported here, no toxicities have been observed in the skin of the mice over several months. Multiple tissue studies showed no organ damage or inflammation due to MWCNT injection [16]. Substantial studies must be accomplished to determine specific and/or nonspecific toxicities for these nano materials before their use in humans.

6.2 Conclusion

Multi-Walled Carbon Nanotubes (MWCNTs) exhibit novel physical properties which makes them ideal candidates of minimally-invasive and non-invasive thermal agents of LITT. Combined with real time 3D temperature imaging MRTI, the results of a small animal experiment show that the growth of a mouse kidney tumor is significantly inhibited by single low-power NIR (only 3W/cm², 30 seconds). Laser irradiation can be controlled by using MRTI as a non-invasive monitor. This monitored thermal treatment could minimize potential injuries to the healthy structures surrounding the tumor and provide real-time feedback during a treatment session. An 80% tumor regression was observed in the MWCNTs group mice. As a comparison, tumor regression was not seen in the control group or the saline group. The difficulty of the experiment is to control the size of the tumor. Since the laser beam for the experiment is not steerable, tumors with a larger size than the laser beam could not be treated thoroughly. For example, the mouse in Fig. 5.5.2.a&b has a tumor of 2cm in diameter which exceeded the diameter of the laser beam (1cm). As shown by the MRTI result, the laser heats only half of the tumor and the rest of the tumor is still at normal temperature. In this case, the tumor may have an incomplete response or recur due to the inadequate treatment. Ideally, for the future, a more advanced laser system needs to be built so that a physician could control the laser
direction and angle or change the laser beam size to heat the whole tumor according to the MRTI.

The successful MRTI-guided-MWCNTs-LITT experiment indicates that this new method could be applied to human superficial tumor thermal treatment non-invasively or minimally-invasively. Several other nanomaterials are being studied for their potential application of cancer therapy, for example, metallic and carbon-based nanomaterials, such as SWCNTs [81], carbon nanohorns [82], gold nanoshells [83], and nanorods [84]. MRTI guided LITT described in the thesis could also use these thermal agents.

Besides using MWCNTs as an NIR absorber and heating generator, these nanotubes could also be used as molecular shuttles for chemotherapeutic agents [85, 86] radionuclides [87], nucleic acids [88] and diagnostic agents [89]. We observed that Fe containing MWCNTs is also a potential effective T₂ weighted MR CAs whose R₂ value is five times higher than the clinically MR CA, Feridex. Thus, tumor could be located by Fe containing MWCNTs. Furthermore, the precise locations of these heating sources could be used in simulating heating results in a computer planning system before the treatment. The drawback of using the Fe containing MWCNTs is that its short T₂ relaxation leads to a void signal in the MR image. As a result, temperature data cannot be calculated in the region where Fe containing MWCNTs are located. However, MRTI could still provide the temperature distribution near the injection site of Fe containing MWCNTs. Additional experiments need to be done to test the accuracy of temperature measurement in the presence of these Fe containing MWCNTs, because the ferromagnetic iron particle will affect the B₀ field.
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Awards and Professional Society

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  - **X. Ding***, R. Kraft, A. Bruke, D. Carroll, S. Torti, J. Bourland *Magnetic Resonance Temperature Imaging Guided Laser-Induced Thermal Therapy with Multi-Walled Carbon Nanotubes.*

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- Varian OBI CBCT/kV
- Positron Emission Tomography
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- Stereotactic Radiosurgery (Novalis Brainlab, Elekta SBRT Body Frame, GammaKnife) & (Elekta Perfexion GammaKnife)

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