Development of a Phantom for Calibrating Thermal Therapy Devices Using MRI

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
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University of Toronto

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0-612-46059-2
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Thermal coagulation therapy aims at treating tumors with high temperatures in the range 60-90 °C. A critical requirement for effective therapy is that the heat pattern from heating devices must be matched to the tumor's contours as accurately as possible. For this matter, new devices that generate heat patterns suited to treat tumors of irregular shapes are being developed. These devices, however, need to be calibrated and tested prior to their use in therapy. This thesis describes the development of a phantom material designed to characterize heating devices in terms of the spatial extent of thermal response they produce. The material records thermal patterns from heating devices by coagulating at high temperatures and can be imaged using magnetic resonance imaging (MRI) to depict these patterns after a heat treatment is delivered to the phantom. Experiments were conducted that characterize its MRI properties with temperature and demonstrate its ability to record thermal patterns from microwave heating devices. Calibrations of this sort can be used for quality assurance, comparison of devices, or for designing and testing new devices.
Acknowledgments

Thanks to the numerous people who have helped and/or supported me. These include, in random order, and hopefully without omissions: Mike Noseworthy, Robert Peters, Greg Stanisz, Leo Zan, Rennie Tang, Jeff Stainsby, Rajiv Chopra, Normand Konyer, Jae Kim, Simon Graham, Mark Henkelman, Graham Wright and Peter Ottensmeyer. Additional thanks to my advisor Mike Bronskill for his criticism, and for teaching me the art of communicating a scientific idea into words. Thanks to my parents for their constant support and love.

This project was supported by a Terry Fox Program Project Grant of the National Cancer Institute of Canada.
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3.1 Temperature dependence of $T_1$ and $T_2$ of a 5% ribonuclease solution ................................. 39
"Thermal coagulation therapy" is the coagulation of tissues using high temperatures in the range 60 - 90°C for periods of seconds to a few minutes to achieve cell death. This approach is to be contrasted with low-temperature thermal therapy (hyperthermia), where tissues are elevated to 40 - 45°C for longer times (several minutes to hours), usually as an adjuvant to radiation therapy or chemotherapy. Radio-frequency (RF) heating, also termed RF ablation, is an example of thermal coagulation therapy. It was introduced by Kirschner [1] and Bauer [2] in the 1930s as a method to coagulate Gasserian ganglia. Since then, there has been great interest in using high temperatures as a stand-alone method for treating various lesions such as tumor masses. This has led to the development of heating devices capable of heating surface or deep-seated lesions, as well as asymmetrical lesion shapes.

Because of their ability to generate large amounts of power, heating devices must be well characterized from the viewpoint of patient safety and treatment planning and delivery. Treatment planning for thermal coagulation therapy demands that the heat pattern generated by these devices be matched to the tumor's three-dimensional (3D) contours. It is thus necessary to characterize devices according to the geometry of their thermal patterns. The situation in vivo, however, is complicated by the dynamical effects of blood flow, intrinsic biologic variability of tissue properties among subjects, and the variation of these properties upon heating [3,4]. For these reasons, it is unlikely that treatment planning can be done to an accuracy capable of predicting the exact outcome of a treatment. It is conceivable that the delivery of heat treatments will be done in conjunction with methods, such as imaging, to monitor the progress of therapies. There is nonetheless great interest in "calibrating" these thermal therapies prior to treatment.

The interest emanates from several perspectives. First, considerable information can be gained from a knowledge of the 3D distributions of thermal effects that a given device produces. Since the real treatment requires matching the heat pattern to the tumor's 3D contours, the shape of the pattern will be one of the first indicators to
determine if a particular treatment is suited to a specific clinical situation. Devices that
do not produce the required shape can be ruled out. Secondly, certain devices may
have different operating parameters that need to be optimized for producing a suitable
heat pattern. For example, an ultrasound phased array probe may require setting the
pulse amplitude, frequency or the relative phase delays among array elements. It may
be necessary to test and compare the effects of different operating parameters. Third,
there is a need from a practical standpoint for the technical maintenance and quality
assurance of clinical devices. Finally, there is interest in developing and testing new
heating devices.

Most of these issues can be addressed with the use of phantom materials. In
radiation therapy, phantoms are physical models commonly used for the quality
assurance and maintenance of radiation therapy equipment, or for simulating a patient
undergoing a treatment. The situation for thermal coagulation therapy is similar except
that the treatment method is heat, and therefore phantoms that have suitable thermal
properties must be used. This thesis illustrates the feasibility of using a material that
coagulates when heated. The delivery of a thermal treatment to the material using a
heating device produces a change in its properties that can be imaged following
treatment, enabling characterization of heating devices in terms of the thermal
response they produce. The material does not simulate all of the complex properties of
tissues and hence cannot be relied upon to predict the outcome of a real treatment, but
instead it provides a standard way to assess 3D thermal response patterns for a given
device. Qualitatively, the shape of the pattern can identify the advantages and
disadvantages of a heating device. Quantitative information from these patterns can
also be used to design new heating devices, to test and compare heating devices, or to
compare the effects of varying their operating parameters.
The phantoms that have previously been used to test and characterize heating devices fall into two categories:

1. Phantoms designed to measure temperature elevation effects such as the specific absorption ratio (SAR) pattern [5]. The SAR pattern describes the rate of energy deposition of a heating device, which is a useful measure of its heating capabilities. These are usually tissue-equivalent phantoms designed to match closely the absorption properties of tissues such as their dielectric properties in the case of RF and microwave heating. The method typically requires temperature measurements to be made during, or immediately following the delivery of a short burst of energy to the phantom using the heating device. The rate of energy deposition is a function of the time it takes to cause an incremental change in temperature. The SAR can be related to thermal damage if the temperature distributions and heating time are known.

2. Phantoms to assess thermal damage. These include real tissues, such as excised animal organs, used with artificial vessel perfusion to simulate the cooling effects of blood flow in tissues [6]. Thermal damage patterns can be assessed visually or by histologic analysis by carefully slicing the organ following the delivery of a treatment, an approach that is not always accurate and/or reproducible. Excised tissues such as liver can be untidy if used in the MRI environment and have a short lifetime. Furthermore, their properties are not constant over time and exhibit broad variability among animals. These phantoms therefore cannot be used quantitatively in comparative experiments.

Egg-white has also been used to assess the extent of thermal response [7]. Unfortunately, fluid motion can perturb the integrity of the pattern, and the method is not quantitative.

This thesis introduces a gel phantom that records thermal damage and which is easy to prepare. Its properties can be standardized. For quality assurance purposes, the phantom is practical, readily available, and easy to use in rapid checks. If used in comparative studies, or for research and development, this phantom provides data which are accurate.
1.1 Image-Guided Therapy

Technological breakthroughs in medical imaging have led to significant advances in the use of imaging methods to guide treatments. These include fluoroscopy, computed tomography (CT), ultrasound, and MRI. Image guidance systems are typically used to locate the position of surgical tools relative to anatomical structures, and to visualize the anatomic structures that lie beneath the surface of the body. An important motivation for using image guidance stems from the potential to improve the accuracy and lessen the invasiveness of interventional procedures [8]. For example, the surgical resection of a brain tumor requires an open craniotomy to expose the target tissue. This invasive procedure can result in patient morbidity while the actual resection often results in physical damage to the surrounding tissues. By allowing the surgeon to view the position of his instruments relative to the contours of the tumor, intra-operative MRI can help in performing more accurate resections, thereby improving the outcome of the intervention. Image guidance also enables less invasive procedures to be performed with the use of smaller openings to access the target tissue, with smaller catheters and other smaller instruments.

In thermal coagulation therapy, small interstitial heating devices such as microwave antennas can be used to generate heat in a tumor. Due to the previous lack of methods to monitor the delivery of energy, the clinical use of these therapies has been hampered. By providing thermographic images or assessments of heat-induced tissue damage to monitor the progress of therapy, image guidance methods such as MRI offer new hope for using these smaller and less invasive devices.

A number of image guidance systems are already in use. X-ray fluoroscopy has been used to guide transcatheter closure of atrial septal defects [9], the placement of pacemakers [10] and biopsy needles [11], to guide fluid drainage procedures [12], fallopian tube recanalization [13] and a number of vascular interventions [14]. X-ray computed tomography (CT) is sometimes preferred over fluoroscopy when good spatial accuracy is required. Unlike fluoroscopy, CT systems in general do not provide a real time display but are nevertheless useful when used intermittently during the procedure.
Examples of these procedures include transthoracic biopsies and abscess drainage [12], and more recently, the percutaneous removal of osteoid osteomas [15]. Continuous exposure to x rays subjects the patient and surgical team to unwanted doses of radiation [16,17], making the use of ultrasound or MRI preferable when equivalent results can be obtained. Ultrasound systems have been used to guide a variety of procedures including thermal ablation of tumors [18,19], cryosurgery [20,21], biopsies [22], intralesional drug administration [23], minimally invasive surgery [24,25] and coronary interventions [26].

MRI provides good contrast for distinguishing different types of soft tissues and can image in any scan plane. Recent advances in MRI technology have led to "Open MRI" units designed to guide interventional procedures. Open MRI units, unlike conventional MR imagers which lack space to do surgery, include a gap to provide surgical access to the patient [27], as shown in Fig. 1.1. MRI has been used to guide a variety of procedures, including neurosurgery [27], minimally invasive surgery [28], biopsies [29], thermal coagulation therapy [8] and vascular interventions [30].

Figure 1.1 MR image guidance system at Toronto General Hospital - Western Division (IGMIT System, GE Medical Systems Inc., Waukesha, WI). Prior to surgery, the surgical team is carefully aligning the patient for imaging. This system allows the patient to be imaged between the two magnet poles, while the surgeon has good access to the patient's head from the opposite side (not shown).
1.2 Thermal Coagulation Therapies

As mentioned previously, thermal coagulation therapies require that high temperatures, 60 - 90°C, be generated to achieve cell death. To generate heat, one can use techniques that rely strictly on thermal conduction, such as hot water circulation or hot wire systems. Hot water systems have the advantage of simplicity. Since the process relies on heat conduction, however, only limited penetration through tissue can be achieved. The heat pattern is typically characterized by maximum temperatures near the applicator, falling off rapidly with distance, as illustrated in Fig. 1.2. Ultrasound [31], lasers [32], microwave [33], and RF [8] heat sources have the ability to deliver energy at a distance from the applicator. The temperature gradients of an RF applicator are compared to a hot source in Fig. 1.2. The greater penetration depth of RF applicators over hot sources offers the possibility of treating deep-seated tumor masses. If necessary, one can move the applicator to expand the region of thermal damage to cover the entire tumor. These methods are of particular relevance in thermal coagulation therapy of tumors that are deep-seated, or that have irregular shapes.

![Figure 1.2 Sketch of radial temperature gradients comparing an RF applicator and thermal conduction hot source. The heating parameters here are hypothetical (for illustrative purposes only) and could be assumed to have similar heating times. The main idea is that heating at greater depths can be achieved with RF hot sources. These characteristics can be employed to generate focal regions of thermal damage. Increased heating depth can also be achieved with laser, microwave or ultrasound heat sources.](image-url)
Effective treatments require temperatures between 60° and 90 °C. They must be high enough to cause coagulation and low enough to avoid boiling or vaporization. To minimize collateral tissue damage, the heat pattern should be matched to the tumor’s 3D contours. Despite the fact that heating devices can be designed to treat specific tumor geometries, it is nevertheless necessary to monitor or control the progress of therapy. This is due to the substantial variability in thermal and physical properties of tissues among subjects, which results in thermal lesions of varying sizes and shapes [3,4]. Modern image guidance systems are likely to be used to monitor temperature elevations and/or heat-induced tissue damage. By using intra-operative images the physician could, for example, decide whether or not enough energy was delivered. Such image guidance systems could be used in a feedback process to monitor and possibly control therapy. Several methods have been investigated to monitor thermal coagulation therapy, including ultrasound, CT and MRI [8,19,34,35]. At the present time, it is likely that MRI will be the method of choice [8,36].

1.2.1 RF Ablation

RF ablation was introduced in the 1930s by Kirschner [1] and Bauer [2], but the method was rapidly discredited due to technical problems and complications of the procedure [37]. It was later demonstrated that the application of RF current directly on the lesion resulted in more effective treatments than by application of RF current through the skin layer [38]. This technique of RF ablation is now well established in the field of neurosurgery [39], for the treatment of brain tumors [40] and Parkinson’s disease [41]. It has also been used to treat liver cancer [42] and for the removal of osteoid osteomas [15]. A common approach uses capacitive coupling with electrodes placed on the target. An RF current is driven at frequencies in the range 500 kHz -27 MHz, producing heat through ohmic losses (collisions of free electrons with atoms and molecules). Temperatures of 80 - 90°C can be generated in a few seconds. When necessary, an array of electrodes can be used to produce diffuse thermal patterns that are well suited for treating large tumors. RF electrodes of any shape and size (e.g. needles or electrode plates) can be designed to treat specific tumor geometries.
1.2.2 Focused Ultrasound Surgery

High-intensity focused ultrasound (HIFU) surgery is a method to produce an extremely localized deposition of acoustic energy. It was used during the 1940s and 1950s for the selective destruction of brain tissue in behavioral studies, and is used today in the treatment of Parkinson's disease [43]. HIFU has also been used for lithotripsy [44], breast tumors [45], thermal coagulation of benign prostatic hyperplasia [46], liver tumors [47], and ocular melanomas [48]. HIFU can be delivered noninvasively when applied extracorporeally by focusing the ultrasound beam into a very narrow region, while sparing surrounding regions and tissues between the transducer and the target site [49]. Jolesz's group in Boston has performed a number of procedures using HIFU [31,50,51,52] where in vivo they were able to create lesions as small as 2 mm in diameter, demonstrating the potential of HIFU to treat very small tumors as well as other diseases that require such localized treatment. Very few tumors that are detected, however, are as small as 2 mm in diameter. Fortunately, ultrasound transducers of virtually any shape can be built to target larger or irregularly-shaped tumors. Interstitial applicators are also of interest for coagulating tissues that lie beneath bony structures such as the skull [53].

1.2.3 Microwave Coagulation Therapy

Microwave radiation (300 - 2,450 MHz) can also be used to cause coagulation in vivo. Microwave coagulation therapy has been used to treat liver tumors [54], bone tumors [55], for the ablation of the adrenal gland [56] and cardiac accessory pathways [57], and is used to treat benign prostatic hyperplasia [58,59]. The microwave radiation field of a heating device interacts by exerting forces on the electric charges present in the medium. These forces can polarize atoms and molecules. Heating is a consequence of energy transfer to the vibrational modes of atoms and molecules [60]. Devices such as helical and coaxial antennas placed directly into the target tissue have been used in most of the papers quoted above. External application of microwaves to treat deep-seated tumors is difficult because microwaves are rapidly attenuated by tissues and are reflected by the subcutaneous fat. Deeper penetration can be achieved at the expense
of narrower thermal patterns by using lower frequencies (e.g. 200 MHz).

1.2.4 Interstitial Laser Therapy

The use of lasers to coagulate tumors was first proposed in 1983 by Brown [61]. The procedure known as interstitial laser therapy (ILT) makes use of relatively low power light (1-20 Watts) with wavelengths in the range 400 - 1024 nm to generate heat through the deposition of energy into the target tissue. The beam of coherent light is delivered through optic fibers placed percutaneously or directly onto the target organ. Fiber tips in general have a frosted coating to diffuse the laser light at wider angles. This design is believed to result in a lower risk of carbonizing tissue near the fiber which could otherwise affect the delivery of energy [62]. ILT has been used to treat a variety of soft tissue tumors including liver metastasis [19], cerebral tumors [32], breast tumors [36], nasopharyngeal tumors [63], and head and neck tumors [18]. It may also be possible to use ILT as a direct continuation of biopsies by inserting optic fibers through a biopsy needle into the tumor [8].

1.3 Motivation for Developing a Phantom

Phantom materials for thermal therapies are needed that perform several functions: heating device characterization, comparative studies of devices, quality assurance and technical maintenance of devices, and research, improvement and development of new devices. Most existing phantoms were developed for clinical hyperthermia and lack one or several requirements for use in thermal coagulation therapy. Phantoms for SAR measurements are useful to understand the behavior and control of power-delivering equipment but often lack the spatial accuracy necessary to measure 3D thermal patterns. Excised tissue or egg-white phantoms can be used to measure thermal response but the results are not always accurate.

This thesis introduces a phantom material which can help perform several of the aforementioned duties. By coagulating at high temperatures, it can record thermal response patterns accurately. Such a method of characterization of heating devices in
terms of thermal effect has the advantage of being simple and direct. Phantoms can be made readily available for use in rapid checks, for testing devices, for quality assurance, for optimization of heating protocols, and for studying the shape of thermal patterns. The material has suitable properties that enable imaging of thermal patterns by use of MRI. Given the likelihood that thermal therapies will be done under MRI guidance, it makes sense to design phantoms that can be used to calibrate the various components of a clinical system used for these therapies.

Advance knowledge of the thermal damage pattern for a specific device can help in planning treatments by enabling the physician to rule out devices that do not produce the required shape. Additionally, devices such as phased array applicators that produce variable heat patterns can now be calibrated and optimized. This was not previously possible in a quantitative way.

Chapter 2 describes the development and characterization of the Zerdine™-BSA material, tested with a microwave heating device. Chapter 3 discusses ways to adapt the material for related purposes. Parts of this work were presented as a poster at the 39th Experimental Nuclear Magnetic Resonance Conference held in Asilomar, California in March 1998. Chapter 2 was presented as a poster at the 7th Annual Meeting of the International Society of Magnetic Resonance in Medicine held in Philadelphia, Pennsylvania in May 1999 and was submitted to Medical Physics in July 1999.
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Chapter 2  MR Imaging of Thermal Coagulation Effects in a Phantom for Calibrating Thermal Therapy Devices

2.1 Abstract

A material has been developed and tested that permanently records thermal response patterns from heating devices. The material consists of a mixture of polyacrylamide and 20% w/w bovine serum albumin. Thermal denaturation is complete when the local temperature exceeds 70 °C, causing a large reduction in the $T_2$ of the material. Three-dimensional distributions of “thermal damage” can be assessed using standard MR imaging sequences. The material works well with microwave heating devices and is adaptable for use with ultrasound, radio-frequency or laser devices. Suggested uses include characterizing heating devices prior to their use in treatment and developing new clinical applications for thermal therapies.

2.2 Introduction

Tissue coagulation by heat has been known for several decades [1,2] but the widespread clinical use of thermal coagulation therapies has been hampered by the lack of methods to control the delivery of heat. Effective treatments require that the entire tumor volume be heated to 60 - 90 °C to induce rapid cell death; any residual tumor mass can result in regrowth of a tumor. To deliver controlled heat patterns at sufficient depth in tissue, several methods have been considered, including lasers [3], RF [4], ultrasound [5] and microwave heating [6]. The most appropriate method depends on the tumor size and shape as well as its location. In recent years, MRI has been established as a promising method to monitor tissue temperature and possibly help control the delivery of heat [7,8]. Studies to characterize the MR properties of
tissues during and after heating are underway [9]. Advance knowledge of thermal damage patterns from different heating devices is desirable for planning safe and effective thermal treatments. There are few phantoms, however, adequate for measuring the spatial extent of thermal damage from heating devices prior to treatment.

A type of material has been developed that records thermal damage patterns from heating devices. The material "coagulates" when the local temperature exceeds 70°C, resulting in a large change in $T_2$. Following the delivery of a thermal treatment, the phantom can be imaged by use of MRI to depict the three-dimensional (3D) distribution of thermal damage with good accuracy. The material consists of a mixture of Zerdine™ polyacrylamide (CIRS Inc., Norfolk, VA) [10] and a concentrated solution of bovine serum albumin (BSA). Thermal denaturation of BSA serves to create coagulation effects [11,12] while Zerdine™ provides a stable matrix that allows solid gel phantoms with appropriate properties to be prepared in various shapes.

The MR properties of a 10% BSA solution were investigated to determine if the changes in $T_1$ or $T_2$ caused by thermal denaturation are suitable for mapping thermal damage. Changes in $T_1$ or $T_2$ determine the amount of contrast that can be obtained in MR images. Further thermal experiments were conducted to characterize the MR properties of the Zerdine™-BSA material itself. Larger changes in $T_2$ of the material make $T_2$ the better parameter for mapping thermal damage. Finally, MR imaging experiments that involved heating a phantom of Zerdine™-BSA with an interstitial microwave antenna were conducted.

2.3 Methods

2.3.1 NMR Relaxation Measurements

Initial experiments consisted of measuring the MR properties $T_1$ and $T_2$ before, during, and following thermal denaturation of a BSA solution. A 10% concentration of heat-shock fractionated BSA (BSA Fraction V, Sigma Chemical Co., St. Louis, MO) was dissolved in phosphate-buffered saline (PBS) solution and stored at 8 °C until needed.
Other experiments established that this concentration represented a good compromise between achieving sufficient denaturation to provide a strong signal change with heating and the difficulty of dissolving high concentrations of BSA. Sodium azide (0.6% w/w) was added to prevent bacterial growth.

$T_1$ and $T_2$ were measured as functions of temperature during a continuous thermal cycle from 22 °C to a peak temperature and back, in steps of 3 °C. At each step an interval of 50 seconds was allowed for heating (or cooling) and stabilization, followed by a 30-second $T_1$ and $T_2$ measurement. $T_1$ and $T_2$ were measured using a three-point inversion recovery sequence followed by a 2000-echo Carr-Purcell-Meiboom-Gill train (90° RF pulse followed by a series of 180° RF pulses) with a 90°-180° interpulse spacing of 0.5 ms. Samples of BSA solution of approximately 0.3 cc were cycled from 22 to 79 °C and back in steps of 3 °C. The consequences of incomplete denaturation were investigated using lower peak temperatures, of 61, 64, 67 and 70 °C. Similar samples of the Zerdine™-BSA material of approximately 0.3 cc were cycled from 22 to 82 °C and back. All experiments had an average heating/cooling rate of 2.25 °C/min.

NMR experiments were performed with a 20-cm horizontal bore 1.5 T superconducting magnet (Narolac, Martinez, CA) equipped with a SMIS spectroscopy console (SMIS, Surrey, UK). The signal-to-noise ratio (SNR) for these samples following a 90° pulse was always greater than 1000, without signal averaging. The 90° pulse lengths were typically 12 µs. Measurements using the 30-second $T_1$ and $T_2$ sequence were in good agreement with slower but more accurate control measurements made before and after each thermal cycle. The calculations of $T_1$ were corrected for temperature-induced systematic errors due to imperfect flip angles for the 90° and 180° pulses throughout the thermal cycle. All relaxation curves were mono-exponential within experimental error. Our home-built temperature control system consisted of a UP750 unit (Yokogawa Corp., Tokyo, Japan) regulating the temperature of an airstream circulated around the sample. A heater element and tap water cooling system was used to increase or decrease the temperature of the airstream. A Luxtron™ thermometer system (Luxtron™ 3100, Luxtron Corp., Santa Clara, CA) measured the sample temperature and was used for feedback to the temperature.
controller unit. The temperature cycling system maintained control within 1°C of the temperature set.

2.3.2 Microwave Heating Experiments

Several disc-shaped phantoms were prepared by pouring a mixture of Zerdine™ and a PBS-buffered 20% w/w BSA solution into cylindrical molds made from 2.5 cm-long segments of plastic pipe (5 cm inner diameter). The phantom ends were sealed with plastic wrapping and a side filling port enabled filling the phantom. The Zerdine™ polyacrylamide was chosen because it is MR-compatible and maintains a high water content (up to 90% w/w) [10]. Zerdine™ could also be chosen to characterize ultrasound heating devices, as its ultrasound properties (attenuation, speed of sound) can be adjusted to mimic tissue.

The imaging experiments were designed to map distributions of thermal damage due to microwave heating. A helical microwave antenna was used (Domier Medical Systems Inc., Kennesaw, GA), which is known to produce an ellipsoidal heat pattern with radial symmetry [13]. The helical emitter tip of the antenna was inserted at the center of the phantom disc, normal to the outer surface. The tip of the antenna was positioned at mid-depth of the disc's thickness. The antenna was connected to the Domier microwave generator and amplifier operating at 915 MHz which was placed outside the MR imager room to minimize interference. The antenna was connected to the amplifier through a low-loss coaxial line running through a penetration panel. Microwave power (~20 Watts) was applied for 10 minutes, then the phantom was allowed to cool for 15 minutes.

$T_2$-weighted images were acquired at room temperature before and after heating (spin-echo, TR = 3000 ms, TE = 70 ms, field-of-view (FOV) = 8 x 8 cm, 5-mm slice thickness, matrix = 256 x 128, 4 signal averages), with the imaging plane centered at half-depth through the disc. MR imaging was done on a 1.5 T whole-body imager (Signa Advantage 5.6, GE Medical Systems, Waukesha, WI) using a birdcage knee coil. The SNR, measured as the ratio of the average signal intensity in a region of
interest centered on the phantom to the standard deviation of the background in air, was at least 100.

To establish a threshold temperature corresponding to thermal damage, dynamic MR temperature images were acquired during heating. The MR thermometry technique employed in this study is based on the temperature sensitivity of the proton resonance frequency (PRF). The PRF of water protons is linearly proportional to temperature over the range 20 - 90 °C; a temperature change results in a shift in the proton resonance frequency of approximately -0.01 ppm/C°. After waiting a time TE following an RF pulse, the temperature-dependence of the proton resonance frequency results in a phase accumulation which is proportional to the shift in resonance frequency. By subtracting the phase angle of the MR signal at a given time to the corresponding phase angle at room temperature, one obtains a phase difference measurement which is directly proportional to the change in temperature. In this experiment, one hundred 15-sec images were acquired over 25 minutes. The relevant parameters were: fast spoiled-gradient echo sequence, \( \theta = 30^\circ \), TR = 50 ms, TE = 20 ms, FOV = 8 x 8 cm, matrix = 256 x 128, 5-mm slice thickness, 2 signal averages. Prior to heating, a baseline phase reference image was obtained by averaging ten images acquired at room temperature. The baseline image was subtracted from each subsequent phase image to obtain a series of phase-difference images. The difference in phase angles was converted to temperature using a coefficient of -0.01 ppm/C° [14], which corresponds, for TE = 20 ms, to a decrease of 4.6 phase angle degrees for a 1 C° increase in temperature. Corrections for phase drifts during the experiment were made relative to an agar gel reference phantom placed within the FOV of the experiment and kept at room temperature. Three Luxtron™ probes were used to provide additional independent temperature measurements throughout which were in good agreement with the MR-derived temperature measurements.
2.4 Results

Fig. 2.1 summarizes the effects of thermal denaturation on $T_1$ and $T_2$ in BSA solutions and Zerdine™-BSA material. The 10% BSA solution denatures at temperatures between 55 and 70 °C resulting in an irreversible decrease in $T_1$ and $T_2$. The degree of denaturation markedly increases with peak temperature up to 67 °C, but little beyond, suggesting a threshold temperature of ~70 °C for complete thermal damage. After heating to this temperature and cooling, the sample’s $T_1$ decreases by 20 % from its value prior to heating while $T_2$ decreases by ~80 %. This change is irreversible as shown by the hysteresis in the curves. The Zerdine™-BSA material has its $T_1$ decreased by 8% and $T_2$ by 80%. The larger percentage decrease makes $T_2$ a more suitable parameter than $T_1$ for mapping thermal damage in these materials.

A post-heating $T_2$-weighted image shows clearly the central region of thermal damage surrounding the antenna (Fig. 2.2). The dark region of thermal damage has a diameter of 8 ± 0.6 mm. The uncertainty in identifying the dark pixels was estimated to be one pixel on each side of the pattern, each pixel having a width of 0.3 mm. For the imaging parameters used here (spin-echo, TR = 3000 ms, TE = 70 ms), the signal intensity in the dark region is decreased by a factor of 5 compared to the unheated material. Because the region of intermediate intensity extended over 3-4 pixels, the pixels were judged to be dark when the intensity was decreased by more than half. This high contrast due to the large decrease in signal intensity is well-suited to map intricate thermal damage patterns.
Figure 2.1

NMR measurements of $T_1$ (a) and $T_2$ (b) as functions of temperature. Samples of BSA solution and Zerdine™-BSA material were cycled from 22 °C to a peak temperature and back. The peak temperatures were 79 °C (■), 70 °C (●), 67 °C (▲), 64 °C (▼) and 61 °C (●) for the BSA solutions and 82 °C (X) for the Zerdine™-BSA material. Thermal denaturation of these samples occurs in the 55-70 °C region, as indicated by the decrease in $T_1$ and $T_2$. Curves ■, ● and X illustrate the effects of full denaturation. The error bars are 2% of $T_1$ and 0.5% of $T_2$ and are smaller than the symbols. This is discussed in more detail in Chapter 3.
Post-heating $T_2$-weighted image displaying the central region of thermal damage surrounding the antenna. The signal intensity in the thermal damage pattern is fivefold lower, over a circular region of 8 mm diameter, compared to undamaged regions. On the left is an agar gel reference used for MR thermometry purposes. The Luxtron™ temperature measurements of the first probe, which were made at a radial distance of about 5 mm from the antenna, remained within 1% of the MR-derived temperature.
The post-heating $T_2$ pattern was compared with peak temperatures reached during heating in order to establish a threshold temperature corresponding to thermal damage. A map of peak temperatures was calculated from the full set of dynamic MR temperature images (illustrated in Fig. 2.3) and compared to an approximate $T_2$ map (spin-echo, TR = 3000 ms with echoes at TE = 14, 50, 100, 200ms, 2 NEX) on a pixel-by-pixel basis. Based on the results of Fig. 2.1 which indicated that the lowest $T_2$ occurred at a temperature of approximately 70 °C, the pixels that exceeded 70 °C in the map of peak temperatures were turned red and overlaid on the $T_2$ map. The region where the temperature was ≥70 °C corresponded to the region of low $T_2$, with $T_2$ less than 50 ms, which appeared as a fivefold decrease in signal intensity, to an uncertainty estimated at one pixel on either side of the damage pattern. The diameter of the red pattern was 8 mm ± 1 pixel when measured in any direction, with a 0.3mm pixel size. For illustration purposes, Fig. 2.4 compares post-heating $T_2$ values with peak temperature along the line shown in Fig. 2.2. The dashed lines are a projection of the 70 °C threshold temperature on the $T_2$ values. These lines indicate that the 70 °C threshold correspond to very low $T_2$ values (below 50 ms). This lowered $T_2$ property is responsible for the decreased signal intensity due to the thermal response.

The phantom was carefully sliced in half approximately 2 hours after heating and a white pattern corresponding to the location of the thermal damage could be seen (Fig. 2.5). The width of the white pattern is ~8 mm, in agreement with the $T_2$-weighted data.

### 2.5 Discussion

Thermal coagulation effects can be captured in a gel material consisting of a mixture of 20% BSA solution and Zerdine™ polyacrylamide. Unlike some other gels, the preparation of Zerdine™ does not involve heating. Although the polymerization of acrylamide is an exothermic process, the associated temperature rise is insufficient to result in premature denaturation of BSA [10]. The material can be used to fabricate phantoms to simulate complex anatomic structures.
Figure 2.3

MR temperature images at various times after the start of microwave heating. The microwave power was on for 10 minutes, then the phantom was allowed to cool for 15 minutes. The agar gel reference is on the left.
Comparison of $T_2$ with peak temperature for a profile along the line shown in Fig. 2.2. The region of thermal damage surrounds the microwave antenna ($r=0$) and has a lower $T_2$ value (32 ms) in a 8-mm-wide region, corresponding to a threshold temperature of 70°C, as indicated by the dashed lines. Intermediate $T_2$ values near the boundaries of the thermal damage pattern (4 mm < $|r|$ < 6 mm) reflect partial denaturation. Phase artifacts immediately adjacent to the antenna (hatched region) are not shown.
Figure 2.5

Photograph of the phantom disc cut in half after heating directly showing the region of thermal damage. The white region has a width of ~8 mm, in the direction of the MR image plane.
The material worked well with a microwave heating device due to its high water content and the electric conductivity of the PBS buffer. The material was not tested with laser or RF heating devices but it should be possible to adjust its properties to accommodate these heat sources. The dielectric properties at radio-frequencies could be adjusted, for example, using the methods described by Chou [15] while absorption of laser light can be adjusted by adding India ink. This is discussed further in Chapter 3. To characterize ultrasound heating devices, the ultrasound properties of Zerdine™ can be adjusted to mimic tissue [10].

\(T_2^*\)-weighted MR imaging following heating provides a depiction of 3D distributions of thermal damage. The spatial accuracy ultimately depends on imaging resolution and the sharpness of the edges of the pattern. Edges are primarily due to the partial denaturation effects exemplified in Fig. 2.1 which result in smaller \(T_2\) changes, provided the voxel sizes are kept small enough to avoid significant partial volume effects. In Fig. 2.4 the edges are less than 2 mm wide in a slice thickness of 5 mm with 0.3 x 0.3 mm pixels. Smaller voxel sizes can be used at some expense in SNR, which can be regained by signal averaging. This can be done with no restrictions on total scan time because, once denatured, the material shows no further changes over several days unless it is re-heated. This was verified by relaxation measurements involving re-heating each BSA solution using an additional thermal cycle. There was no further change in \(T_1\) or \(T_2\) unless the peak temperature in the second thermal cycle exceeded that of the first.

The shape of the curves in Fig. 2.1 can be explained as follows. Outside the denaturation transition, between 22° and 55 °C, and beyond 70 °C, \(T_1\) and \(T_2\) have an approximately linear dependence on temperature. This is consistent with \(T_1\) and \(T_2\) being inversely proportional to the correlation time, \(\tau_c\), of water molecules (i.e. \(1/T_1\), \(1/T_2^*\)). The correlation time of a large ensemble of molecules is defined as the average time between successive molecular reorientations. Higher temperatures are a consequence of water molecules that have higher kinetic energy, and a shorter correlation time. Since the correlation time is inversely proportional to temperature (\(\tau_c \propto 1/T\)), both \(T_1\) and \(T_2\) of rapidly-moving water molecules are directly proportional to temperature (\(T_1, T_2 \propto T\)).
The decrease in $T_1$ and $T_2$ between 55° and 70 °C is explained by realizing that both water and protein molecules have their own $T_1$ and $T_2$. These differ by several orders of magnitude. For example, the relaxation times of water are of the order of seconds while those of proteins are in the microseconds range. The very short $T_1$ and $T_2$ of proteins make it impossible to observe the protein signal directly. However, the presence of proteins affects the water signal by enhancing the relaxation of water molecules through cross-relaxation [12]. Proteins expose labile protons that interact with water molecules, altering the $T_1$ and $T_2$ of water. In the case of $T_1$, dipolar couplings and chemical exchange between water and protein protons mediate this process [16]. $T_2$ cross-relaxation is modulated by chemical exchange, or the physical transfer of hydrogen atoms between sites of different resonance frequency [12].

At approximately 55°C, BSA unfolds to an enlarged conformation, causing a slight reduction in the tumbling speed of BSA molecules. The reduced tumbling speed of BSA may cause the $T_1$ and $T_2$ of water to decrease via cross-relaxation. Unfolding also results in more exposed protein protons. This would cause an increase in the number of sites that mediate cross-relaxation, and hence a decrease in both $T_1$ and $T_2$. In Chapter 3, results on a solution of ribonuclease, for which heat-induced unfolding occurs, suggest that only $T_2$ is affected by this process (Fig. 3.1). Therefore, the decrease in $T_2$ of water must be due to a lower protein $T_2$. The unfolding of BSA, however, is followed by an aggregation reaction, which results in immobilization of the protein molecules. The $T_2$ of proteins is significantly reduced (from hundreds of microseconds to a few microseconds [12]), while the protein $T_1$ is increased slightly [12]. The much reduced protein $T_2$ is a significant contribution to the large decrease in water $T_2$ via cross-relaxation [12]. The observed decrease in $T_1$ has been attributed to an increase in the rate at which cross-relaxation occurs [16]. A possible explanation for this is as follows. The extensive protein gel network may have, on average, a reduced distance between its constituting protein fragments compared to the non-denatured case. This means that the distance that water molecules must travel before reaching a protein surface to be relaxed is shorter. During the time required to make a $T_1$ or $T_2$ measurement, this diffusion-mediated relaxation process is more effective when the macromolecular surfaces are closer to each other, as in the case of thermally-denatured BSA. This concept applies to both $T_1$ and $T_2$. 

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2.6 Conclusion

A material has been developed and characterized that records thermal damage patterns from heating devices. The material, a mixture of Zerdine™ polyacrylamide and BSA protein that is solidified into a gel form, can be used to prepare phantoms of various sizes and shapes to simulate organs. Complete thermal denaturation occurs by 70°C and results in a change in the $T_2$ of the material which can be mapped using standard MR imaging sequences. Because the heat-induced changes are permanent and reflect the cumulative history of the heating protocol, MR imaging can be done anytime after treatment without risk of changes in the thermal damage pattern. The material can be used to characterize heating devices prior to their use in treatment, to test heating protocols, for quality assurance, and to develop new clinical applications for thermal therapies.
References


Chapter 3  Discussion

3.1 Material Uses and Properties

This thesis illustrates the feasibility of using phantoms made of Zerdine™-BSA material to characterize heating devices in terms of thermal response. This is of interest to understand the behavior and control of heating devices, which can have several inherent operating parameters [1]. This phantom material can also be used to improve, develop and test new heating devices, and to characterize and optimize heating protocols. If the material is used for technical maintenance or quality assurance purposes, the performance of a clinical system can be determined under standardized conditions, with a reduced number of variables.

Liquids such as egg-white lack the accuracy of gels because fluid motion can perturb the integrity of the thermal damage pattern. The Zerdine™-BSA material contains a polyacrylamide (Zerdine™) that holds the material in solid gel form and withstands temperatures up to 100 °C. Furthermore, convection currents which perturb temperature distributions are significantly reduced in solid gels. The resulting matrix makes it possible to prepare phantoms that preserve the integrity of thermal damage patterns. This is an important feature for assessing thermal damage with good accuracy. In a real treatment, it is desirable to avoid temperatures that are too high. Our phantom does not show further changes in $T_2$ beyond 70°C. Therefore it does not tell if the temperature was too high in parts of the region of thermal damage. This phantom should not be used to predict the temperature elevations in vivo.

The material does not simulate the complex heterogeneous properties of tissue. It does not preclude the need for monitoring the progress of therapy by such means as, for example, imaging. From the standpoint of planning treatments, one must be aware that the results are likely to be different in vivo. For example, blood perfusion has regional cooling effects while large blood vessels act as local heat sinks. Moreover, the
variability of tissue properties among subjects results in variable lesion sizes. The Zerdine™-BSA phantom can help identify the advantages and disadvantages of a specific device, or it can be used to optimize the operating parameters of a device. It must not, however, be used to predict the outcome of treatments in vivo as real tissues include inhomogeneities such as fat, bone, air pockets, large blood vessels that would cause the actual thermal patterns to be distorted. Thermal therapies should instead be done in conjunction with guidance methods that include checkpoints where the thermal effects can be assessed. At these checkpoints, the physician will likely decide whether or not enough energy was delivered. This permits adjustments to the heating regime to be made.

To understand the mechanisms of heat delivery, the study of the effects of tissue inhomogeneities on thermal patterns is of interest. The presence of inhomogeneities tends to affect the delivery of energy and thermal conduction. For example, ultrasound suffers from its inability to traverse gas cavities such as occur in the lung or bowel, and it is readily reflected and absorbed by bone. The reflection of ultrasound at air-muscle or bone-muscle interfaces can result in standing wave patterns that interfere constructively to create hot spots at the interface. Similarly, electromagnetic radiation can result in hot spots at muscle-fat interfaces. The presence of bone and air cavities, whose thermal diffusivities differ significantly from those of soft tissues, can affect the heat conduction process. For tumors located close to a bone, the greater thermal insulation exhibited by the bone restricts the flow of heat into it, and can therefore result in hot spots [2]. These hot spots are of great importance due to the high heating potential of certain devices. Large blood vessels also play an important role in tissue heat transfer and the resultant temperature distributions [3].

1. The thermal diffusivity, \( \alpha \), defined as \( \alpha = k / \rho c \) (\( k \) = thermal conductivity, \( \rho \) = density, \( c \) = heat capacity) is used as a compact notation to summarize the characteristics of heat flow in a medium. (Good heat conductors have a high thermal diffusivity value whereas insulators have a low value.)
Simple experiments can be done that involve simulating thermal treatments on heterogeneous phantoms. In situations where the tumor lies near fat, bone, air or large blood vessels, such simulated treatments would help optimize the heating protocol. Phantoms of Zerdine™-BSA may be cast that include bones, air cavities, fluid-carrying channels, or fat layers into a mold shaped to the anatomy of interest. Such phantoms would help in understanding the effects of inhomogeneities on the shape of thermal patterns. Additionally, feedback can be used to design heating devices aimed at treating tumors located near inhomogeneities.

For different heating sources, the energy absorption properties of the material must be adjusted so that a reasonable amount of heating can be produced. Insufficient heating can result in small thermal damage patterns that are more difficult to characterize. Overheating and exaggerated heat patterns must be avoided because the subsequent flow of heat by thermal conduction could blur out the contours of thermal damage patterns. The precise matching of absorption properties with those of tissues is not necessary. However, to achieve heat patterns that are reasonable in size, the absorption properties of the material must lie in the vicinity of those of tissues.

The dielectric properties of Zerdine™-BSA (as described in chapter 2) in the microwave frequency range can be estimated as follows. The dielectric constant (\(\varepsilon'\), the real part of the complex permittivity) of tissues is determined by their high-permittivity water content (\(\varepsilon'=78\) for water at 22 °C [4]) and the fraction of proteins which tend to decrease the permittivity [5]. The magnitude of this decrease in tissues at microwave frequencies is typically 20-30 dielectric units, for protein contents of 15 to 25% of the total tissue weight, yielding permittivities between 50 and 60 for most high-water-content soft tissues. The electric conductivity (\(\sigma\)) is due to the various electrolytes in solution and varies between 8 and 16 mmho/cm in the frequency range 100 - 915 MHz for tissues with a high water content [6]. In comparison, the Zerdine™-BSA material contains roughly 85% saline water for which \(\varepsilon'=78\) and \(\sigma=18\) mmho/cm [4]. The protein content leads to a dielectric decrement of the order of 1 unit/g protein/100 ml of water [5]. For a protein concentration of 20 g protein per 100 ml water, this results in a decrease of 20 dielectric units in \(\varepsilon'\). The effects of Zerdine™
polyacrylamide and other additives such as ethylene glycol, which comprise approximately 10% of the total weight, were not investigated but may cause a small decrease in permittivity. Dielectric properties do not vary substantially at microwave frequencies up to 1 GHz [7]. Thus the properties are probably close to tissues with \( \varepsilon' = 58 \) and \( \sigma = 18 \text{ mho/cm} \). Even though an exact match is not required, the dielectric properties can nevertheless be adjusted with the proper ingredients [7]: adding proteins decreases the permittivity, and sodium chloride increases conductivity.

Diverse biological materials with high water content have heat capacity and thermal conductivity values close to those of pure water [8]. The thermal conductivity and heat capacity of polyacrylamides is very close to tissues, even at high acrylamide concentrations (up to 35 percent of total weight) [9]. It is expected that, because it contains mostly water, the thermal properties of Zerdine™-BSA are reasonably close to tissues.

Thermal therapy does not limit itself to using microwave heat sources. Heat sources such as RF, ultrasound, and lasers need to be characterized and tested for the same reasons as those mentioned above. The material can be adapted to accommodate these heat sources as well: the dielectric properties of the material at radio-frequencies can be adjusted according to Chou² [6]; the acoustic attenuation is adjusted by varying the alumina or boron nitride content, and the speed of sound by changing the concentration of ethylene glycol or turpentine oil [10]; the optical absorption is increased by adding India ink and erythrocyte concentrates, and scattering is adjusted with silica powder and Intralipid [11].

2. For example, aluminum powder increases the dielectric constant, whereas polyethylene powder decreases it; sodium chloride increases the electric conductivity.
Finally, a word about the reproducibility of the results in Chapter 2 is necessary. All NMR measurements were repeated several times on different days. The microwave heating experiment was repeated twice on different days. In all cases, the results did not change. The random error in the measurements of $T_1$, $T_2$ (Fig. 2.1), as determined by repeated measurements on the same sample, were 2% of $T_1$ and 0.5% of $T_2$. Several BSA solutions were prepared identically and there was no detectable variability in $T_1$ or $T_2$.

The sample-to-sample variability of the results on Zerdine™-BSA was not addressed in this thesis. All of the phantoms used were made using a single preparation of Zerdine™-BSA, which was manufactured directly by CIRS Inc in November 1997. In a private communication with CIRS Inc. it was revealed that a PBS-buffered 20% w/w BSA solution was used, and that the acrylamide was prepared according to Fig. 13 of their patent [10]. Fig. 13 of the patent indicates that the acrylamide concentration is 8 g per 95 g of BSA solution (or 8.4% of the total weight). The assessment of sample-to-sample variability is of fundamental importance to the reproducibility of the results involving Zerdine™-BSA. It would be necessary to investigate this matter further.
3.2 Developing a Reversible Phantom

When Zerdine™-BSA is heated once, the induced changes are irreversible. The main advantage of using an irreversible phantom is that thermal damage can be recorded permanently, and can be imaged anytime after heating. Without posing any restrictions on imaging time, high-quality images can be obtained with good spatial resolution. The fact that the material can be used only once represents a disadvantage. If large quantities of phantom are required for quality assurance checks to be made on a regular basis, the costs incurred may be considerable. In 1999, the cost to manufacture Zerdine™ was approximately $300 per kilogram. A kilogram may be good for about 10 experiments. The price of BSA is $1000 per kilogram of powder, for an additional cost of $100 to manufacture one kilogram of Zerdine™-BSA. A more cost-effective approach is possible if the phantom can be re-used several times. A material would be re-usable if the heat-denatured protein content can renature back to the original state.

Several proteins and polypeptides exist that renature upon cooling, with minimal aggregation. These include ribonuclease [12], methionine repressor protein from E. Coli [13], myosin protein fragments [14], C16 serine protease fragments [15], wild-type kringlet-2 [16], spectrin single-motif peptides [17], S-protein [18], and Arc repressor [19]. These proteins denature upon heating and revert back to their original state after cooling. If these proteins are present in sufficient concentrations (millimolar range), the denaturation of such proteins should produce a change in $T_2$ upon heating. In some of the above papers that reported complete reversibility, the experiments were done at physiological concentrations in the micromolar range. These small concentrations are unlikely to produce any significant change in $T_2$. As the concentration increases, the likelihood of aggregation between unfolded polypeptides also increases. Therefore, it may be that some of these proteins do not renature completely or aggregate to form precipitates when present at millimolar concentrations. Preliminary results on ribonuclease, however, seem to suggest that reversibility at high concentrations occurs.
The MR properties $T_1$ and $T_2$ of a 5% w/w ribonuclease solution were measured as functions of temperature. The solutions were prepared by dissolving 0.5 g of ribonuclease powder (ribonuclease A, Sigma Chemical Co., St. Louis, MO) into 10 ml of sodium formate buffer. The sodium formate buffer maintained a pH around 4.0 after dissolution of ribonuclease. A low pH value provides favorable conditions for denaturation and minimal aggregation; however very low pH values (<3) can cause unfolding of the protein [20]. The measurements of a solution undergoing heating from 22 to 77°C followed by cooling to 22 °C, in steps of 5 °C, are shown in Fig. 3.1. The MR parameters $T_1$ and $T_2$ are seen to revert to their original values after cooling. Thus ribonuclease exhibits good reversibility under those conditions.

Figure 3.1. Heat denaturation of ribonuclease A followed by renaturation. The absence of hysteresis effect between heating and cooling indicates reversibility.
With a reversible phantom, an important concern in imaging thermal patterns is to image at the right time. According to Fig. 3.1, a post-heating MR image would not reveal any changes because the solution renatures after cooling, losing memory of the thermal effect. MR imaging needs to be done during heating. This would be feasible in light of the fact that some thermal treatments are delivered in 10 to 20 minutes and that MR imaging can be done in less that 2 minutes, depending on the choice of sequence.

For the thermal pattern to be visible, it should appear with sufficient contrast. A quick estimate of contrast for a 5% ribonuclease solution without polyacrylamide can be obtained from the data of Fig. 3.1. The $T_1$ and $T_2$ values at the 22°C starting point and at 77°C, the hypothetical end point where $T_2$ is lowest, are $T_1 = 1800$ ms, $T_2 = 1150$ ms, and $T_1 = 3100$ ms, $T_2 = 1050$ ms, respectively. Using an equation of the form $S = S_0(1-\exp(-T/T_1))\exp(-T/T_2)$ with $T_e = 1000$ ms and $T_f = 2000$ ms to describe the MR signal intensity, the resulting contrast enhancement would be $2(S_{22°C} - S_{77°C})/(S_{22°C} + S_{77°C}) = 40\%$. This amount of contrast should be sufficient to permit mapping of thermal patterns. This contrast enhancement is not solely due to the change in $T_2$, but also includes a contribution from $T_1$ which increases substantially. If the contrast due to $T_1$ were absent (as in the case of a strictly $T_2$-weighted scan), the contrast enhancement would be only 8% and the thermal pattern would be hard to depict. Thus by carefully choosing the imaging parameters, adequate contrast enhancement could be obtained that permits mapping of thermal patterns. In a phantom, however, the effects of the polyacrylamide and interactions with other Zerdine™ ingredients (ethylene glycol, boron nitride, glass microspheres, polyethylene powder, alumina, phenolic microspheres, graphite, oils) on the reversibility of protein renaturation need to be considered.
In 1999, the market price of ribonuclease was $260 per gram of ribonuclease A, for a cost of $13,000 to manufacture one kilogram of Zerdine™ containing a 5% ribonuclease solution. To match the low cost of Zerdine™-BSA, a single phantom would have to be re-usable at least 32 times. This alternative would fit nicely in the context of thermal therapy where image guidance during treatment could be useful. For example, this would provide an opportunity for testing rapid $T_2$ imaging sequences, which are likely to play an important role in guiding therapies. Evidently, the main advantage of irreversible denaturation that allows imaging to be done anytime after heating would be lost.

Another option which appears less feasible, but could be considered at a later time when progress in protein engineering is at a more mature stage, is to use heat-shock proteins (hsp) to renature an otherwise irreversibly-denatured phantom. The hsp’s act by preventing aggregation of denatured proteins and, under the right conditions, can assist the refolding of proteins [21]. Unfolded proteins that expose hydrophobic residues from their core are subject to forces driving the aggregation process, unless the hydrophobic residues are allowed to interact with a chaperone surface [22]. The denaturation process becomes irreversible when proteins aggregate among each other to form cross-linked polymer networks. The KJE chaperones from *E. coli* (DnaK, DnaJ and GrpE) are believed to act in concert to restore the full enzymatic activity of aggregation-prone proteins [23]. DnaK can bind denatured proteins and peptides to prevent their aggregation [24], while the cochaperones DnaJ and GrpE act synergistically to stimulate DnaK in assisting the refolding of denatured polypeptides into active proteins [25,26]. This chaperone-assisted refolding process is, however, ATP-dependent. Using this fact, Diamant and Goloubinoff [23] have successfully restored the enzymatic activity of thermally-denatured proteins in protein solutions containing chaperones by using a refolding buffer containing ATP. The proteins were, however, present at micromolar concentrations, and this would be insufficient to produce a resolvable change in $T_2$. 
The renaturing buffer used by Diamant and Goloubinoff [23] contains pyruvate kinase (PK), an enzyme whose function is to convert ADP back to ATP in order to maintain high levels of ATP at all times. If a gel phantom (containing protein and chaperones) were to be soaked in such a renaturing buffer, the PK molecules would have to diffuse into the material, a process that may be seriously hampered by the gel matrix. On the other hand, if instead of preparing the renaturing buffer with PK, PK was initially incorporated into the material as an ingredient, it would risk being irreversibly inactivated during heating.

This option would be feasible only if it can be demonstrated that chaperones can renature denatured proteins efficiently at millimolar concentrations, and that the energetics of the ATP hydrolysis reaction can be well sustained. This means that the process must be "fueled" for a period of time sufficient to renature a significant proportion of proteins present in the phantom, unless chaperones are used that do not require ATP. The presence of the gel matrix must not interfere negatively with this process. Finally, it must be demonstrated that sufficient change in $T_2$ can be obtained with the protein-chaperone combination, especially in light of the fact that macromolecular binding of chaperones to denatured proteins could possibly overshadow the well-appreciated relaxation effects of denatured proteins on $T_2$. 
References