A phantom feasibility study of acoustic enhanced drug diffusion in neurological tissue

George K. Lewis Jr.*
Department of Biomedical Engineering
Cornell University, 108 Olin Hall,
Ithaca, NY 14850
george@cornellbme.com

Dr. William Olbricht
Department of Chemical Engineering
Cornell University, 108 Olin Hall
Ithaca, NY 14850
Wlo1@cornell.edu

Abstract--To test the feasibility of using 1.1MHz focused ultrasound to increase the diffusion of drug through neurological tissue, tissue mimicking agar phantoms and a water soluble dye mimicking drug were exposed to 1.1MHz ultrasonic sound fields for 1 to 4 minutes in duration. Histology was then performed on the phantoms to assess the effect of ultrasound on the perfusion of drug. Mathematical analysis was used to quantify the extent of perfusion of dye and parametrically fit the experimental data to the theoretical diffusion equation.

Results show a substantial increase in drug perfusion and not diffusion like behavior for the sonicated phantoms.

I. INTRODUCTION

Designing effective therapies for brain cancer, including neuroblastoma, glioblastoma, and neurofibromatosis remains a significant challenge. Despite the development of drugs that are effective against these malignancies, the prognosis for patients remains poor. One reason for the poor outcome is that malignant cells can infiltrate healthy tissue surrounding the tumor, leading to poorly defined tumor boundaries. Migrating cells not only escape removal during tumor resection, but they may also avoid radiation and chemotherapy after resection. As a result, the tumor regrows, usually aggressively, and often at a location close to the site of the original tumor [1 and 2].

To address these issues new drug delivery methods have been developed to circumvent the blood-brain barrier and deliver drugs directly to brain parenchyma. Gliadel wafers impregnated with BCNU are implanted into the resection cavity to deliver high concentrations of drug to the surrounding tissue. However, the diffusivity of BCNU in tissue limits its penetration to a few millimeters from the wafer before it is eliminated by a variety of mechanisms [3]. This penetration distance is insufficient to reach more distant malignant cells. Similar considerations apply in convection enhanced delivery, a technique in which drugs are infused directly into the brain through a needle. Near the infusion site, transport is dominated by convection, but the strength of convection decays with distance from the infusion site and diffusion becomes important distant from the infusion needle [4 and 5]. Therefore, a method to increase the diffusion of compounds in tissue could enhance the therapeutic outcome of these drug delivery methods.

Acoustical techniques have been used in a variety of situations to enhance brain cancer treatments. For example, high intensity focused ultrasound (HIFU) has been used to ablate and liquefy neurological tissues, and past and current studies are being conducted to assess the use of HIFU as a more complete surgical tool for minimally invasive therapy [6, 7 and 8]. Focused ultrasound is beginning to be assessed as a feasible way to deliver drugs to neurological tissues via selective disruption and permeablization of the blood brain barrier (BBB). Drugs that once could not cross the BBB because of molecular weight and hydrodynamic radius are now able to permeate into the neurological tissue with the application of ultrasound [8 and 9]. Ultrasound has also been used in other applications such as gene therapy and drug activation, and for further information on therapeutic ultrasound the authors cite [8].

In this study we explore the use of US to increase the perfusion of locally delivered drug into an agarose gel that is used routinely as a mimic of brain tissue for drug delivery studies. The ultimate goal is to increase the rate of transport of pharmaceutical agents relative to their elimination rate, and thereby extend the distance that drugs penetrate and maintain therapeutically useful concentrations.

* This work was supported by the Cornell University Life Science Initiative, National Science Foundation Fellowship and Transducer Engineering INC.

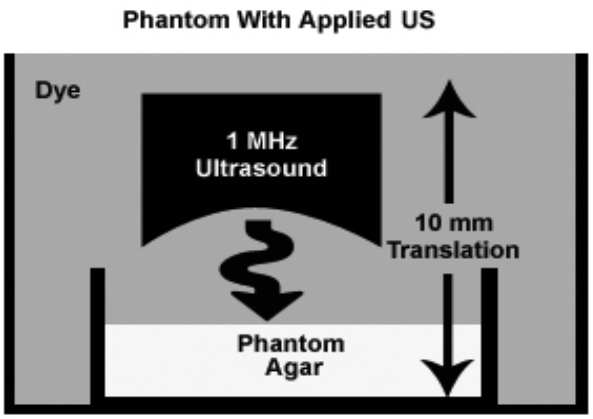

Fig. 1. The experimental setup for the phantom study is shown. The sonicated phantom is immersed in 0.5 wt% diluted dye and pulse sonicated (15 seconds on and off) while simultaneously the transducer is oscillated up and down at 0.25Hz 10mm amplitude. The beginning position of the transducer was 40mm above the agar/dye interface, so that the US focus was placed on the surface. The control setup was the same without US.

II. METHODS

Neurological tissue mimicking phantoms were prepared by filling standard 85 x 15 mm plastic Petri dishes with a solution of 0.6 wt% agar powder in warm distilled water [10]. The dishes were then set aside to allow the solution to gel. Red food coloring, diluted in distilled water to 0.5 wt% was used to mimic water soluble drug and apply contrast to determine the extent of perfusion. Ultrasound (US) energy in the phantoms was generated by a lead zirconate titanate (PZT-4), 1 MHz, 20mm diameter piezoelectric ceramic with a radius of curvature corresponding to 40mm (EBL Products Inc., Connecticut, USA). The ceramic, housed in a PVC plastic assembly, was driven at its center harmonic frequency, determined to be 1.1 MHz, by a +/- 200 volt, 55 Ohm, RF amplifying circuit. The circuit was constructed from Supertex Inc., TC6320 high speed switching MOSFET's. The RF driving circuit was supplied from a function generator (HP 8116A, HP USA) with a sinusoidal 1.1 MHz waveform. It was experimentally determined that the transducers input impendence was 65 Ohms at resonance, determined by measuring the voltage across the US transducer when attached to a 50 Ohm 10 V 1.1 MHz function generator source. A 95% acoustic conversion efficiency was estimated (as a high value) from supplied manufactures values. Measuring the amplifiers output voltage with and without the US device attached, knowing the US device impeadence and using voltage division with the conversion efficiency of the ceramic a total acoustic output power was estimated to be 4.5 watts.

The experimental setup shown in figure 1, consisted of the control and the sonicated phantoms. The phantoms and dye where allowed to equilibrate to room temperature before the experiment. The phantoms were immersed in 500 ml of the dye and secured in position with clamps. Phantoms were sonicated on and off (15 seconds each) at their geometric center for durations of 1-4 minutes. The location of the focus of the transducer was positioned so that the focus at 40mm from the front surface of the US was placed on the agar/dye interface. The US was manually oscillated at 0.25 Hz over a 10mm translation to increase the sonicated volume (Figure 1). This was done for the duration of all experiments. For analysis purposes the location of the phantom/dye interface was taken to be zero. Histological readings were taken every minute for the control and sonicated phantoms. Histology on the phantoms was preformed by taking a 1mm geometric center slice from the phantom and imaging it with a ccd camera/microscope system (Nikon Cool Pix 995, Nikon Inc. USA and Olympus BX51, Olympus Inc. USA). The digitally captured image was imported into MatLab 6.5 (Math Works Inc. USA) to determine the extent of the dye perfusion into the phantoms with and without applied US. By using Red-Green-Blue (RGB) color mapping and converting digital pixels into distance (mm) measurements, intensity vs. distance spectral curves of dye perfusion into the phantoms were produced.

We modeled the transport of the dye as one-dimensional diffusion from a source. Experimental data were compared with diffusion profiles for a constant source as shown:

$$N(x,t) = N_0 erfc\left(\frac{x}{2\sqrt{Dt}}\right) \text{(constant source) Equation (1)[11]}$$

where: N_0 is the source concentration,
x is the diffusion distance,
Dt is the diffusion time product and
erfc is the complimentary error function.

Using a least squares approach, we parametrically fit the experimental data to the theoretical equations to compare differences in diffusion between the sonicated and control phantoms.

III. RESULTS

Analysis of the experimental data shows that neurological tissue mimicking phantoms, that were

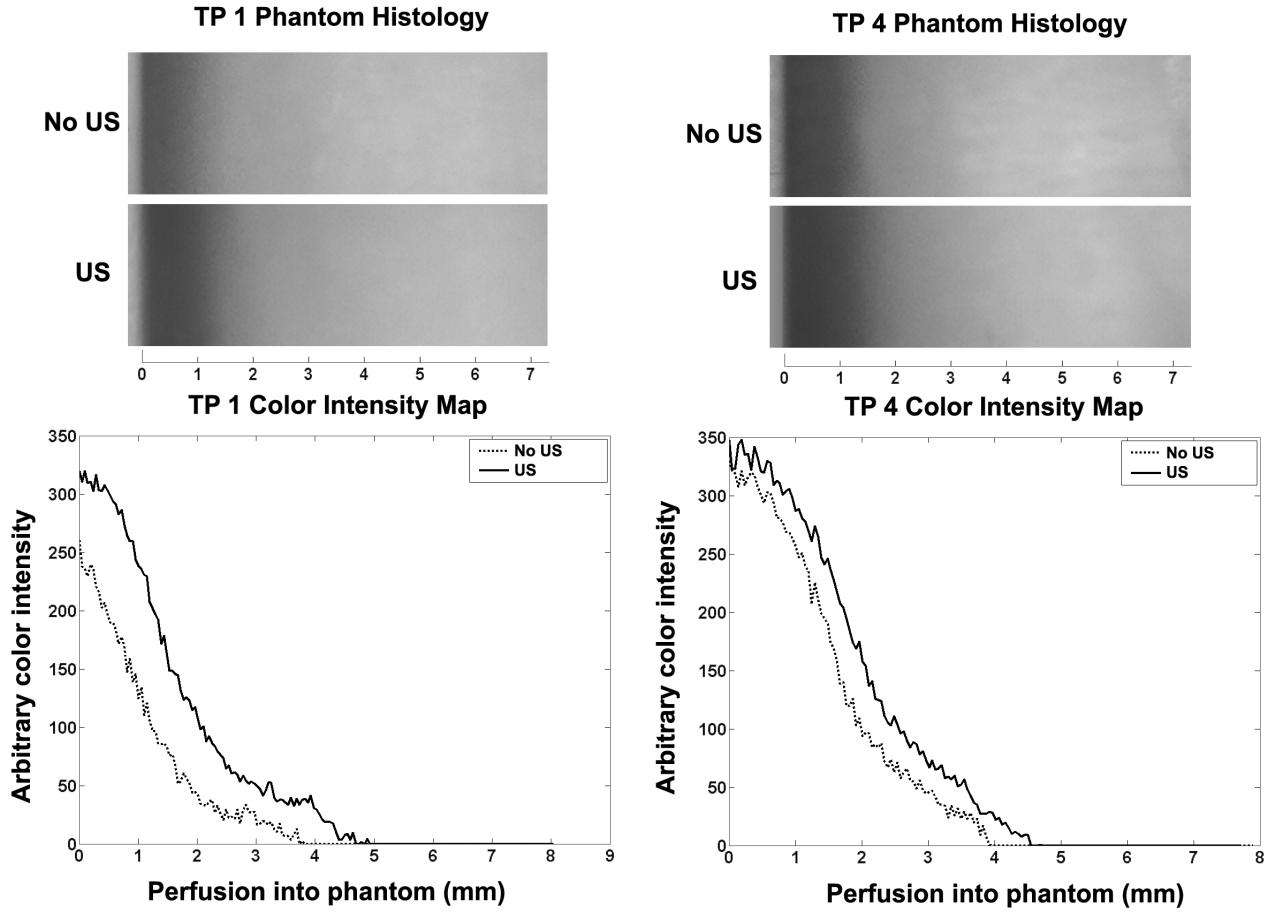

Fig. 2. Top left and right are histology images taken from phantoms with and without applied US TP 1 and 4. Visible is the increase in dye penetration and intensity of the sonicated phantom. Bottom left and right are the corresponding RGB color intensity maps that correspond with the above images.

sonicated, have a substantial increase in dye perfusion when compared to the control. Figure 2 shows experimental results at time points (TP) 1 and 4 minutes with a visual and quantifiable increase in dye perfusion into the sonicated phantoms (similar results for TP 2 and 3 were also quantified but not shown). At 1 and 4 minutes the surface intensity of dye uptake is 25% and 10% greater respectively then the corresponding controls, and the sonicated phantoms show an overall dye uptake increase of 84% and 25% respectively calculated by the difference in area under the color intensity curves at 1 and 4 minutes. Shown in the 4 minute intensity profile of figure 2, we see that the control begins to follow the same shape of dye perfusion as the sonicated phantom at later TP's.

As expected and predicted by theory Eq. (1) we found that the perfusion of dye in the control phantom exhibited model diffusion behavior, TP 1 is shown in figure 3. Also apparent in figure 3 is that Eq. (1) did not predict the experimental results of the spectral data from the sonicated phantoms at TP1. Figure 3 shows that sonication plays a role to increase the depth of dye perfusion and the method in which it moves into the phantom.

During the first time point TP1, figure 2 shows that the surface intensity for the control is not equivalent to the US experiment. A further experiment was conducted by the authors to address this surface anomaly; why the surface concentration of dye in gel for the control phantom did not equilibrate more rapidly. It was concluded that gelling surface properties, most plausibly different cross linking characteristics of the agar, lead to a mass-transport resistance at the surface of the agar phantom. The mass-transport resistance was not apparent when an inner section from the agar phantom was used as the surface for dye to diffuse into. Ultrasound therefore had an impact on breaking down the surface resistance

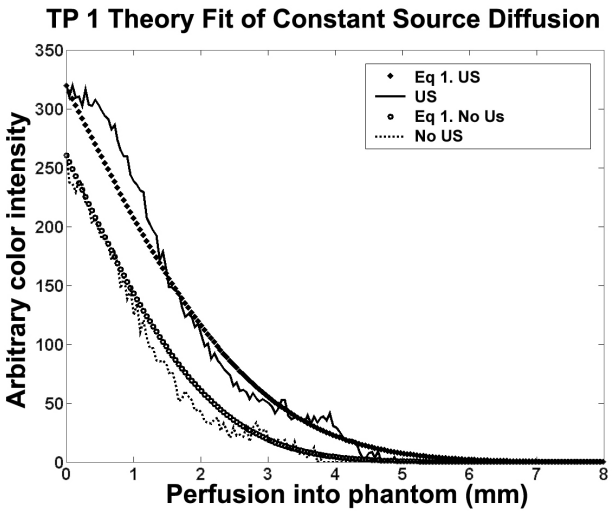

Fig. 3. Plot of the theoretical fit to the experimental data for TP 1. We see good agreement with control (No US) and the constant source diffusion Eq. (1), note that US experimental data does not fit Eq. (1) well.

of the phantom allowing rapid perfusion of dye Figure 2.

IV. CONCLUSION

In this study we looked at the effect of 1.1 MHz ultrasound on the perfusion of dye into neurological tissue mimicking phantoms. We found a clear increase in dye penetration and overall dye density in the sonicated verses control phantoms for all TP's. Most noticeably is the 170% initial increase of spectral intensity from dye uptake at the 1 minute TP as shown in Figure 2, where US was found to increase the surface mass-transfer of dye into agar gel by acoustic radiation pressures and agitation of the agar gel matrix. In the mathematical analysis and parametric fitting of the data we found good agreement of simple diffusion to the control experiments, however simple diffusion processes could not account for the enhanced transport of dye into the phantom with the application of US.

From the results presented we find that 1.1 MHz ultrasound is a promising new method to increase the penetration of hydrophilic drug into brain tissue. The overall goal being the ability to use ultrasound to drive locally delivered chemotherapy agents past current diffusion limitations to reach migratory cancer cells. Further research is planed to study how changes in ultrasound parameters will affect the rate and level of dye perfusion in animal models. Different frequencies, powers, and pulse sequences appropriately below the brain tissue damage threshold will be analyzed to create a therapeutically useful regimen.

ACKNOWLEDGMENT

The authors gratefully acknowledge the contribution of colleagues' and reviewers' comments. We would also like to thank Transducer Engineering INC. for engineering and design support.

REFERENCES

[1] Giese A., Bjerkvig R., Berens M.E. and Westphal M. (**2003**) " Cost of migration: invasion of malignant gliomas and implications for treatment" American Cancer Society Journal of Clinical Oncology, Vol 21, Issue 8 1624-1636, Copyright © Journal of Clinical Oncology

[2] Lefranc F., Brotchi J. and Kiss R. (**2005**) "Possible future issues in the treatment of glioblastomas: special emphasis on cell migration and the resistance of migrating cells to apoptosis" Journal of Clinical Oncology, Vol 23, Issue 10, 2411-2422, Copyright © American Society of Clinical Oncology

[3] Westphal M., Hilt D., Bortey E. , Delavault P., Olivares R., Warnke P., Whittle I, Jääskeläinen J. and Ram Z. (**2003**) "A phase 3 trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant glioma" Neuro-Oncology 5(2): 79-88, Copyright © 2003 Duke University Press

[4] Bobo R.H., Laske D.W., Akbasak A., Morrison P.F., Dedrick R.L and Oldfield E.H. (**1994**) "Convection-enhanced delivery of macromolecules in the brain" Proceedings of the National Academy of Sciences, Vol 91, 2076-2080, Copyright © 1994 National Academy of Science

[5] Vogelbaum M.A. (**2007**) " Convection enhanced delivery for treating brain tumors and selected neurological disorders: symposium review" Journal of Neuro-Oncology 83: 97-109, Copyright © 2006 Springer Science + Business Media

[6] Hill C.R. and Haar G.R. (**1995**) "Review article: high intensity focused ultrasound--potential for cancer treatment" The British Journal of Radiology, Vol 68, Issue 816 1296-1303, Copyright © 1995 by British Institute of Radiology

[7] Vykhodtseva N., Sorrentino V., Jolesz F.A., Bronson R.T. and Hynynen K. (**2000**) " MRI detection of thermal effects of focused ultrasound on the brain" Ultrasound in Med. & Biol., Vol 26(5), 871-880, Copyright © 2000 World Federation for Ultrasound in Medicine and Biology

[8] Mitragotri S. (**2005**) "Healing sound: the use of ultrasound in drug delivery and other therapeutic applications" Nature Drug Discovery, Vol 4, March, pg.255-260, Copyright © 2000 Nature Drug Discovery

[9] Mesiwala A.H., Farrell L., Wenzel H.J., Silbergeld D.L., Crum L.A., Winn H.R. and Mourad P.D. (**2002**). "High-intensity focused ultrasound selectively disrupts the blood-brain barrier in vivo" Ultrasound Med Biol. Mar;28(3):389-400

[10] Chen Z.J and et al. (**2004**) "A realist realistic tissue phantom for intraparenchymal infusion studies" Journal of Neurosurgery 101:314-322, Copyright © 2004 Journal of Neurosurgery

[11] Crank J. *The Mathematics of Diffusion,* Oxford University Press, 1975