Published in final edited form as:

Biochem Biophys Res Commun. 2009 February 6; 379(2): 428–433. doi:10.1016/j.bbrc.2008.12.090.

Activation of transgene expression in skeletal muscle by focused ultrasound

X Lu¹, G Sankin¹, E C Pua¹, J Madden², and P Zhong^{1,*}

¹ Department of Mechanical Engineering and Materials Sciences, Pratt School of Engineering, Duke University, Durham, NC 27708, USA

² Department of Pathology, Duke University Medical Center, Durham, NC 27710, USA

Abstract

To correlate thermal dose from focused ultrasound (FUS) with gene expression and tissue injury, a temperature plateau strategy was employed. Plasmids encoding luciferase gene under the control of hsp70B promoter were transfected into the right gastrocnemius muscle in a rat via electroporation. One day after transfection, hind limbs were treated with 3.3-MHz focused ultrasound, using one of four different temperature plateaus with spatial-peak time-average focal temperatures (T_{SPTA}) of 46°C, 48°C, 51°C and 62°C. The treatment duration at the plateau temperature was varied from 0 to 30 s. Gene expression was analyzed *in vivo* one day following FUS treatment, and H&E staining was employed to assess tissue injury. Gene activation and tissue damage correlated closely with thermal dose. The highest level of gene activation was induced by FUS at $T_{SPTA} = 51^\circ\text{C}$ for 20 s, which was found to be statistically equivalent to that produced by water-bath hyperthermia.

Keywords

Focused ultrasound; gene activation; gene therapy; hyperthermia; heat shock

1. INTRODUCTION

For successful translation to clinical applications, delivery systems for gene therapy must exhibit low systemic toxicity, improved transfection/transduction efficiency, and strong spatial and temporal control over transgene expression [1;2]. However, despite its promise, gene therapy has demonstrated only limited success, in large part because of restrictions in regulation methods. For example, biological regulation of gene expression, such as tissue-specific or disease-specific promoter systems (e.g. EGR1) [3], exhibits strong site specificity, yet is incapable of adequate temporal control and cannot be manipulated externally. On the other hand, chemical regulation methods, such as “Tet-on and Tet-off” [4;5], can achieve good temporal control, but they suffer from a lack of spatial specificity. Physical promotion of gene activation via heat-sensitive systems like the hsp70b promoter, has been shown to provide both temporal and spatial control [6;7]. However, local hyperthermia application typically requires

*Correspondence: Pei Zhong, Department of Mechanical Engineering and Materials Sciences, Pratt School of Engineering, Duke University, Durham, NC 27708, USA. Phone: (919) 660-5336, Fax: (919) 660-8963, Email: pzhong@duke.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

long exposures. Also, many heat sources are invasive or lack fine control in energy deposition for maintaining stable heating patterns *in vivo* [8].

Focused ultrasound (FUS) has recently shown great potential for heat-inducible gene activation due to its focusing ability and high heating efficiency [9;10]. FUS can heat small tissue volumes in deep organs and, if necessary, increase the target temperature rapidly over 90°C within a few seconds [11]. Previous research on heat-induced gene activation employed primarily hyperthermia-level temperatures in the range from 40–44°C for long durations (30–60 minutes). Recently, however, transgene expression from rapid ultrasound heating in the range from 44°C to 50°C has also been demonstrated [12]. Furthermore, we have shown that ultrasound burst mode heating between 55°C to 85°C for 10–30 s can significantly increase hsp70b-luciferase gene expression up to 170-fold in a 4T1 tumor model [9;10]. This suggests that transgene activation may depend on thermal dose rather than peak temperature induced in the target tissue. Thus, shorter treatment times with a high degree of spatial control over heating and gene activation can be accomplished with FUS. However, ultrasound therapy exceeding hyperthermia can also induce thermal damage. Therefore, in the development of improved regulation of gene therapy, it is critical to investigate the relationship between thermal dose, gene activation, and tissue injury produced by FUS.

In this study, we have explored the use of short duration FUS exposures for control of gene activation, employing a temperature plateau strategy that enables a well-defined temperature profile with a controllable duration window for thermal dose evaluation. The aim of this study is therefore two-fold: 1) to determine the correlation between thermal dose, gene activation, and tissue injury *in vivo*, using stable, short duration ultrasound exposure strategies, and 2) to assess the efficacy of rapid FUS heating for gene therapy.

2. MATERIALS AND METHODS

2.1. Animal study protocol

Wistar outbred rats of 250 g body weight were purchased from Charles River Laboratories (Wilmington, MA 01887-1000). All the animal handling procedures were performed in accordance with NIH guidelines, and the experimental protocols were approved by Duke Institutional Animal Care and Use Committee. Prior to all animal procedures, rats were anesthetized by IP injection of 35–40 mg/kg pentobarbital sodium solution (50 mg/ml).

2.2. Ultrasound exposure system

The experimental ultrasound system (Figure 1a) consists primarily of a 3.3-MHz focused ultrasound transducer (Model H-102, Sonic Concepts, Inc., Bothell, WA 98011), driven by a function generator (DS345, Stanford Research Systems, Sunnyvale, CA, USA) and a power amplifier (A150, ENI, Rochester, NY, USA) at a fixed gain of 55 dB. The transducer has a 70 mm active diameter with a geometric focus of 63.2 mm. The pressure waveform and distribution in the focal plane of the HIFU transducer have been measured previously [13]. A custom stage was constructed to facilitate animal handling and positioning, enabling direct placement of the rat's hind limb at the focus of the FUS transducer. Positioning of this stage was performed with 3-D step motors (Velmex, Inc, Bloomfield, NY 14469). Control of the ultrasound transducer system and 3-D step motors was accomplished through a custom PC program, which interfaced with all equipment via GPIB. Experiments were performed in a tank filled with degassed water, maintained at 37°C.

2.3. Exposure strategy and temperature mapping

Multi-step exposure strategies were established to produce stabilized temperature plateaus. These settings were confirmed *in vivo* prior to transfection studies to ensure predictable

temperature control. Specifically, a 0.1 mm bare-wire thermocouple (custom-designed IT-23, Physitemp Inc., Clifton, NJ) was placed through the bore of a 20-G needle and aligned vertically with the tip facing down towards the transducer focus. The thermocouple was conditioned by an electronically compensated isothermal terminal block (model TC-2190, National Instruments Corporation, Austin, TX) and registered at a 2-Hz sampling rate using a PC-mounted data acquisition board (NI4351, National Instrument, Austin, TX). The needle was then used to insert the thermocouple approximately 5 mm into the gastrocnemius muscle of an anesthetized rat. The needle was then retracted from the focal zone, leaving the thermocouple embedded in the target tissue.

Before temperature mapping, the acoustic focal point of the transducer was located through monitoring of maximum temperature elevation during a prescreening process. Low pressure exposures of 5 s duration were used to avoid altering acoustic absorption properties or inducing tissue damage. Mapping was then executed using the multi-step exposure strategies, treating a 4.5 mm $\times$ 4.5 mm region centered about the focus with a step size of 0.5 mm. For each exposure strategy, 3–6 animals were used for temperature mapping. The pressure parameters and their corresponding temperature levels are summarized in Table 1.

2.4. In vivo transgene activation and expression analysis

For transfection studies, Hsp70b luciferase plasmid (a gift from Dr. Dexi Liu, University of Pittsburgh) was prepared with Qiagen endofree kit (Qiagen Inc, Valencia, CA). The final plasmid concentration was 1 μ g/ μ l in 1x PBS. After anesthetization, the gastrocnemius muscle was injected with 400 μ g hsp70b-luciferase plasmid in 400 μ l PBS using four injections of 100 μ l each. Electroporation was immediately applied at the injection site using a BTX electroporator (BTX Instrument Division, Harvard Apparatus, Inc., Holliston, MA 01746-1388) with two needle electrodes at a 0.5 cm gap. Six 40 ms square wave pulses of 200 V/cm amplitude and 960 ms intervals were applied two times to a 0.5 cm $\times$ 0.5 cm square region of the injection area, using a 0.5 cm gap between the two exposures.

One day following electroporation, the transfected skeletal muscle area (9 mm $\times$ 9 mm) was treated by FUS using a raster scan pattern with a step size of 1.5 mm for a total of 49 exposures (Figure 1b). Total treatment time for each subject ranged from 10 to 30 min. One day after FUS treatment, the animal was anesthetized, and 100 μ l D-Luciferin in PBS (15 mg/ml) was injected IM into the treated skeletal muscle. Resultant gene expression was analyzed using an IVIS100 Xenogen imaging system (Xenogen Inc, Alameda, California 94501). Luciferase expression level was detected and quantified as relative light units (RLU) in the entire treated area. Hyperthermia at 42°C for 30 min. in waterbath was employed as a positive control.

2.5. Tissue damage assessment

The procedure described above was applied to a separate group of animals in order to evaluate tissue damage. At 1, 3, 7 and 28 days post-treatment, animals were euthanized. Skeletal muscle tissue samples were then harvested from the FUS-treated site and fixed with 10% formalin. Samples were collected from non-treatment sites in FUS-treated animals and from untreated animals for comparison. Standard H&E staining was performed to evaluate tissue damage. A “histologic injury index” (HII) was developed on H&E stained slices to show the progression of injury and healing in a quantitative manner. Five factors were assessed on a 0 to 3+ scale in the three most severely affected high-power (40x) microscopic fields, generally in triplicate for each condition: fibrosis, regenerative myocyte changes, necrosis and vascular proliferation, polymorphonuclear leukocyte (PMN) infiltration, and mononuclear cell infiltration.

2.6. Thermal dose calculation

To correlate gene expression and tissue damage with the thermal treatment duration and intensity of FUS, thermal dose (TD) was calculated using the following equation [14]:

$$TD(t) = \int_0^t R^{43-T(t')} dt'$$

$$\text{with } R = \begin{cases} 0.25 & \text{if } T(t) < 43^\circ\text{C} \\ 0.5 & \text{if } T(t) \geq 43^\circ\text{C} \end{cases}$$

At 43°C, a treatment duration of 240 minutes was introduced as a reference for thermal lesion formation [15]. All thermal dose calculations were normalized to this standard, with a TD of 1 corresponding to the condition that will lead to protein denaturation and tissue necrosis. It has been shown that this model gives sufficient estimation of thermal lesion formation from FUS [15].

Temperature profiles acquired from the described mapping procedures (Figure 1c) were used to determine the thermal dose delivered *in vivo*. Data from 20 second exposures at 0 mm, 0.5 mm, and 1 mm away from the focus were used for calculations. Thermal dose determination was restricted to the 1 mm × 1 mm region about the focus due to the significant reduction in heat generation outside of this area.

2.7. Statistical analysis

Unless otherwise stated, at least 4 animals were used in each experimental group. The results are shown as mean ± standard deviation, and statistical analysis was performed using the student's t-test.

3. RESULTS

3.1. Targeted heating and temperature distribution in vivo

Figure 1c shows representative temperature profiles produced at the transducer focus using four different exposure strategies (P_I-P_{IV} in Table 1), each comprised of multiple phases. During each exposure, the temperature initially increased rapidly at a rate of 7–16°C/sec for approximately 4 s, reaching a plateau level during the ensuing exposure phases. The spatial-peak time-averaged temperature (T_{SPTA}, °C) in the plateau region was determined to be 46°C, 48°C, 51°C and 62°C, respectively, with a maximum variation of ± 2°C.

The spatial distribution of the peak temperature around the focal point of the 3.3-MHz transducer is shown in Figure 1d. Under each exposure strategy, temperature in the focal plane was found to decrease gradually from the peak temperature at the transducer focus to 37°C at a radial distance of 2.5 mm.

3.2. Transgene activation in vivo

The mean total fluorescence exhibited from treated animals via Xenogen imaging are summarized in Figures 2a and 2b. The data includes a negative control group (C1) which involved animals injected with PBS solution prior to electroporation treatment. The background system noise exhibited from this control was $0.27 \pm 0.07 \times 10^6$ RLU. Positive controls from luciferase plasmid injections with (C3) and without (C2) electroporation are included, with resultant expression levels of $0.70 \pm 0.43 \times 10^6$ RLU and $5.51 \pm 2.64 \times 10^6$ RLU, respectively. Waterbath hyperthermia treatment produced luciferase expression of $30.11 \pm 11.64 \times 10^6$ RLU.

Gene activation levels produced from 20 second exposures (total treatment time = 16–20 min.) of each FUS strategy are compared in Figure 2a. All four strategies produced significantly higher expression levels than controls (C1–C3). The three higher power settings (P_{II}–P_{IV}) exhibited luciferase expression comparable to waterbath treatment for 30 min., although only P_{III} (T_{SPTA} = 51°C) produced an average gene activation ($42.57 \pm 7.42 \times 10^6$ RLU) greater than that of hyperthermia (not statistically significant). These results suggest that, when compared with hyperthermia treatment at 42°C, FUS within the plateau temperature range of T_{SPTA} = 48°C–62°C can produce an equivalent gene activation level with reduced treatment duration.

Figure 2b shows transgene activation levels at T_{SPTA} = 51°C with a single exposure treatment time ranging from 0 to 30 s. With FUS implemented for impulse heating (t = 0 s), luciferase expression doubled from baseline (C3) values to $11.62 \pm 2.41 \times 10^6$ RLU. Gene activation further increased to $22.92 \pm 7.80 \times 10^6$ RLU at t = 10 s, reaching a peak value of $42.462 \pm 12.65 \times 10^6$ RLU at t = 20 s, and eventually dropping to $36.98 \pm 6.64 \times 10^6$ RLU at t = 30 s. In the 10–30 s range, FUS produced comparable gene activation to that of waterbath hyperthermia, with animals treated with 20 s and 30 s exposures exhibiting higher average levels of fluorescence.

3.3. Tissue damage assessment

Based on photomicrographs of H&E stained rat skeletal muscles before and, at successive time points, following FUS treatment (Fig. 3a), injury was observed to be focally marked one day after the treatment in both temperature groups. For T_{SPTA} = 48°C, only minor regenerative changes were evident by the 7th day. These conditions returned to essentially normal histology at 28 days. In contrast, evidence of marked injury and repair was observed after 7 days for T_{SPTA} = 51°C. By 28 days, permanent fibrosis and continuing regenerative changes were present. Injury from electroporation in control groups was observed to be minor.

A “histologic injury index” (HII) was developed to show the progression of injury and healing in a quantitative manner (Figure 3b). The degree of leukocyte infiltration is high for both temperature groups one day after treatment, but regenerative changes are already evident. The HII for T_{SPTA} = 48°C promptly returns to a low value and is indistinguishable from the control by 28 days. By contrast, regeneration is delayed for T_{SPTA} = 51°C, with changes persisting up to the 28-day time point. Fibrosis is evident in the T_{SPTA} = 51°C group, but does not occur for T_{SPTA} = 48°C.

Although tissue changes were evident at T_{SPTA} = 48°C and T_{SPTA} = 51°C, the total damaged portion of the treatment area was less than 2% of theinsonified region. This is consistent with reports on HIFU-induced tissue damage in the same temperature range [16;17]. By contrast, severe tissue ablation at T_{SPTA} = 62°C for t = 20 s was observed in more than 30% of the total treated area (data not shown), suggesting that the tissue damage induced at T_{SPTA} = 51°C was relatively minor.

3.4. Correlation of thermal dose with tissue damage and gene activation

The calculated thermal doses for the four exposure strategies (Fig. 4) used in this study increased exponentially with T_{SPTA} at the focus (r = 0 mm), traversing from slightly below the threshold for heat shock response (P_I) to significantly exceeding the necrosis threshold (P_{IV}). Due to the temperature gradient around the transducer focus (see Figure 1d), TD drops rapidly with radial distance, especially at T_{SPTA} = 62°C.

Overall, the results of the TD calculations support the experimental observations from FUS-induced gene activation shown in Figure 2. Data from Figure 4 suggest that the highest degree

of gene activation should be produced at $T_{SPTA} = 51^{\circ}\text{C}$, with most of the target tissue receiving a thermal dose between the thresholds for heat shock response and necrosis. At lower output settings, a significant portion of the target tissue volume from each FUS exposure will receive a TD below the threshold for heat shock response. On the other hand, P_{IV} greatly exceeds the TD for tissue necrosis, indicating that a lower level of gene activation will be produced.

4. DISCUSSION

Focused ultrasound has the potential to play a significant role in the realm of *in vivo* gene therapy due to its relative safety and efficiency [11;18]. Unlike many hyperthermia applicators, FUS can be delivered non-invasively, with highly specific configurations suited to individual applications. Furthermore, rapid heating from FUS can reduce treatment times with respect to conventional hyperthermia. In this study, we have demonstrated that, under the control of hsp70b-promoter, transgenes in rat skeletal muscles can be sufficiently activated in the spatial-peak time-average temperature range of $T_{SPTA} = 48^{\circ}\text{C}$ to 62°C with a treatment duration of 20 s. In comparison to hyperthermia, a similar gene activation level was observed at the $T_{SPTA} = 48^{\circ}\text{C}$ level with minor injury that could be fully recovered within one week. Further increases in focal temperature led to similar transgene expression levels with sublethal ($T_{SPTA} = 51^{\circ}\text{C}$) to non-recoverable ($T_{SPTA} = 62^{\circ}\text{C}$) tissue injury, suggesting a thermal dose window for transitioning from heat-induced gene activation to necrotic tissue injury. This result has clinical significance when applying heat inducible gene activation in therapeutic applications, particularly for cancer therapy, in which thermal damage provides additional benefits.

A multi-step exposure strategy was employed for the 3.3-MHz focused ultrasound transducer, enabling stable temperature plateaus in rat skeletal muscles. This method differs from our previously described experiment, which utilized short bursts of FUS to reach a peak temperature elevation *in vivo*, followed by an immediate cut-off of the acoustic energy [9; 10]. The strategy employed in this study has the advantage of achieving the desired target temperature increase within a shorter duration, reducing variability between exposures. In addition, the low pressure steps used to sustain the temperature plateau provide a clear window for thermal dose evaluation.

Our results show good correlation between thermal dose measurements and observed experimental results in rat skeletal muscle. The P_{III} strategy provided the most consistent focal zone heating between the hyperthermia and tissue necrosis thresholds, corresponding with the highest level of luciferase expression. Compared with water bath treatment, reduced gene activation may be the result of inhomogeneous heat distribution caused by heat conduction, variable acoustic properties of the treated tissue, and the natural pattern of pressure distribution. Furthermore, elevated temperatures above that of the hyperthermia treatment may initiate cell apoptosis, necrosis, or metabolic change, further affecting the gene activation process. Tissue damage at lower T_{SPTA} values may be caused by temperature fluctuations induced by small variations in tissue properties. Local cavitation may also lead to tissue damage. These comparisons demonstrate that thermal dose can be used as a relative indicator of gene activation; however, the inhomogeneity of heat distribution from FUS combined with the complicated interplay of cell damage and gene expression from sub-lethally heated cells suggests that TD may not be the only predictor of actual gene expression yield.

The small beam size of a HIFU transducer leads to heating of a small tissue volume with a single exposure. This is useful if spatial gene activation is required on a relatively small scale. For large tissue volumes, however, scanning protocols must be applied in order to ensure complete treatment, resulting in longer total treatment times despite short exposure durations at individual scanning points. In addition, the background noise of hsp70b-luciferase gene

expression in this study was noticeably high, which may be caused by tissue damage produced by electroporation during gene delivery [19;20]. In future work, the use of viral vectors may reduce this background noise.

FUS shows considerable promise for gene therapy applications. In particular, site-specific gene activation through heat shock response can be synergistically combined with direct thermal ablation from focused ultrasound [9]. For cancer therapy, coagulative necrosis can destroy bulk tumor tissue while peripheral sub-lethally damaged cells are utilized for gene activation. It will also be valuable to investigate the feasibility of this FUS treatment mechanism in internal organs such as the liver and kidneys. Furthermore, improved thermometric methods have been investigated for more accurate heat deposition and treatment assessment. Using MR-guided FUS systems, improved spatio-temporal control of gene expression can be achieved with direct temperature feedback [8;17].

Acknowledgements

This work was supported in part by NIH through grant #RO1-EB002682. We acknowledge the technical support of W. Neal Simmons and Yufeng Zhou in the construction and calibration of the FUS system.

References

1. Agha-Mohammadi S, Lotze MT. Regulatable systems: applications in gene therapy and replicating viruses. *J Clin Invest* 2000;105:1177–83. [PubMed: 10791990]
2. Pfeifer A, Verma IM. Gene therapy: promises and problems. *Annu Rev Genomics Hum Genet* 2001;2:177–211. [PubMed: 11701648]
3. Lopez CA, Park JO, Mauceri HJ, Beckett MA, Weichselbaum RR, Posner MC. Control of gene therapy by MDR1 and EGR1 promoter sequences in transcriptional targeting by chemotherapy (Review). *Int J Oncol* 2004;24:731–6. [PubMed: 14767560]
4. Gossen M, Bujard H. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc Natl Acad Sci U S A* 1992;89:5547–51. [PubMed: 1319065]
5. Wilson MO, Scougall KT, Ratanamart J, McIntyre EA, Shaw JA. Tetracycline-regulated secretion of human (pro)insulin following plasmid-mediated transfection of human muscle. *J Mol Endocrinol* 2005;34:391–403. [PubMed: 15821105]
6. Brade AM, Ngo D, Szmítko P, Li PX, Liu FF, Klamut HJ. Heat-directed gene targeting of adenoviral vectors to tumor cells. *Cancer Gene Ther* 2000;7:1566–74. [PubMed: 11228535]
7. Rome C, Couillaud F, Moonen CT. Spatial and temporal control of expression of therapeutic genes using heat shock protein promoters. *Methods* 2005;35:188–98. [PubMed: 15649846]
8. Moonen CT. Spatio-temporal control of gene expression and cancer treatment using magnetic resonance imaging-guided focused ultrasound. *Clin Cancer Res* 2007;13:3482–9. [PubMed: 17575210]
9. Liu Y, Kon T, Li C, Zhong P. High intensity focused ultrasound-induced gene activation in solid tumors. *J Acoust Soc Am* 2006;120:492–501. [PubMed: 16875245]
10. Liu Y, Kon T, Li C, Zhong P. High intensity focused ultrasound-induced gene activation in sublethally injured tumor cells in vitro. *J Acoust Soc Am* 2005;118:3328–36. [PubMed: 16334906]
11. ter Haar G. Therapeutic applications of ultrasound. *Prog Biophys Mol Biol* 2007;93:111–29. [PubMed: 16930682]
12. Guilhon E, Voisin P, de Zwart JA, Quesson B, Salomir R, Maurange C, Bouchaud V, Smirnov P, de Verneuil H, Vekris A, Canioni P, Moonen CT. Spatial and temporal control of transgene expression in vivo using a heat-sensitive promoter and MRI-guided focused ultrasound. *J Gene Med* 2003;5:333–42. [PubMed: 12692867]
13. Zhou Y, Zhai L, Simmons R, Zhong P. Measurement of high intensity focused ultrasound fields by a fiber optic probe hydrophone. *J Acoust Soc Am* 2006;120:676–85. [PubMed: 16938956]
14. Sapareto SA, Dewey WC. Thermal dose determination in cancer therapy. *Int J Radiat Oncol Biol Phys* 1984;10:787–800. [PubMed: 6547421]

15. Damianou CA, Hynynen K, Fan XB. Evaluation of Accuracy of a Theoretical-Model for Predicting the Necrosed Tissue Volume during Focused Ultrasound Surgery. *IEEE Trans Ultrason Ferroelectr Freq Control* 1995;42:182–187.
16. Graham SJ, Chen L, Leitch M, Peters RD, Bronskill MJ, Foster FS, Henkelman RM, Plewes DB. Quantifying tissue damage due to focused ultrasound heating observed by MRI. *Magn Reson Med* 1999;41:321–8. [PubMed: 10080280]
17. McDannold NJ, King RL, Jolesz FA, Hynynen KH. Usefulness of MR imaging-derived thermometry and dosimetry in determining the threshold for tissue damage induced by thermal surgery in rabbits. *Radiology* 2000;216:517–23. [PubMed: 10924580]
18. Newman CM, Bettinger T. Gene therapy progress and prospects: ultrasound for gene transfer. *Gene Ther* 2007;14:465–75. [PubMed: 17339881]
19. Edd JF, Horowitz L, Davalos RV, Mir LM, Rubinsky B. In vivo results of a new focal tissue ablation technique: irreversible electroporation. *IEEE Trans Biomed Eng* 2006;53:1409–15. [PubMed: 16830945]
20. Vogel U, Wanner T, Bultmann B. Extensive pectoral muscle necrosis after defibrillation: nonthermal skeletal muscle damage caused by electroporation. *Intensive Care Med* 1998;24:743–5. [PubMed: 9722049]

Figure 1.

Schematic representations of the (A) focused ultrasound system and (B) scanning protocol for gene activation. (C) Average temporal variations in temperature elevation for each ultrasound exposure strategy. (D) Spatial temperature distribution from the 4 exposures settings, measured at the $2.5 \times 2.5 \text{ mm}^2$ focal region.

Figure 2.

(A) *in vivo* hsp70b-luciferase gene activation induced in rat skeletal muscle by hyperthermia and 20 s FUS exposures. (B) *in vivo* hsp70b-luciferase gene activation induced in rat skeletal muscle by hyperthermia and FUS with varying exposure durations at $T_{SPTA} = 51^{\circ}\text{C}$.

A

B

Figure 3.

(A) Tissue histology H&E staining for $T_{SPTA} = 48^{\circ}\text{C}$ and $T_{SPTA} = 51^{\circ}\text{C}$ and $t = 20\text{ s}$ (20x magnification). (B) Tissue damage evaluation at $T_{SPTA} = 48^{\circ}\text{C}$ and 51°C using "histologic injury index" (HII).

Figure 4.

Normalized thermal dose for each experimental FUS exposure strategy at distances $r = 0, 0.5$, and 1 mm from the focal axis. Dashed and dotted lines indicate thermal dose thresholds for tissue necrosis and water bath hyperthermia (30 min. at 42°C), respectively.

Table 1

FUS exposure parameters

Pressure Settings	T _{SPTA} , °C
P _I (2.8MPa, 1s; 2.4MPa, 1s; 1.9MPa, 3s; 1.5MPa, X s)	46
P _{II} (4.35MPa, 0.5s; 3.9MPa, 1s; 2.8MPa, 2s; 2.4MPa, X s)	48
P _{III} (4.6MPa, 1s; 3.7MPa, 3s; 3.2MPa, 5s; 2.8MPa, X s)	51
P _{IV} (6.3MPa, 2.5s; 5.9MPa, 2s; 5.4MPa, 4s; 4.6MPa, X s)	62

X = plateau exposure duration ranging from 0 to 30 s