Published in final edited form as:

Ann Biomed Eng. 2017 December ; 45(12): 2879–2887. doi:10.1007/s10439-017-1926-1.

Increasing Distribution of Drugs Released from *In Situ* Forming PLGA Implants Using Therapeutic Ultrasound

Chawan Manaspon^{1,2}, Christopher Hernandez³, Pinunta Nittayacharn³, Selva Jeganathan³, Norased Nasongkla, Ph.D.¹ [Assoc. Prof.], and Agata A. Exner^{2,3,*}

¹Department of Biomedical Engineering, Faculty of Engineering, Mahidol University, Nakorn Pathom, 73170 Thailand ²Department of Radiology, Case Western Reserve University, 11100 Euclid Ave, Cleveland, OH, 44106 USA ³Department of Biomedical Engineering, Case Western Reserve University, 11100 Euclid Ave, Cleveland, OH, 44106 USA

Abstract

One of the challenges in developing sustained-release local drug delivery systems is the limited treatment volume that can be achieved. In this work, we examine the effectiveness of using low frequency, high intensity ultrasound to promote the spatial penetration of drug molecules away from the implant/injection site boundary upon release from injectable, phase inverting poly(lactic acid-*co*-glycolic acid) (PLGA) implants. Fluorescein-loaded PLGA solutions were injected into poly(acrylamide) phantoms, and the constructs were treated daily for 14 days with ultrasound at 2.2 W/cm² for 10 minutes. The 2D distribution of fluorescein within the phantoms was quantified using fluorescence imaging. Implants receiving ultrasound irradiation showed a 1.7-5.6 fold increase ($p < 0.05$) in fluorescence intensity and penetration distance, with the maximum increase observed 5 days post-implantation. However, this evidence was not seen when the same experiment was also carried out in phosphate buffer saline (pH 7.4). Results suggest an active role of ultrasound in local molecular transport in the phantom. An increase of fluorescein release and penetration depth in phantoms can be accomplished through brief application of ultrasound. This simple technique offers an opportunity to eventually enhance the therapeutic efficacy and broaden the application of local drug delivery systems.

Keywords

controlled release; drug delivery system; PLGA implant; therapeutic ultrasound

Introduction

Drug delivery systems are an essential component of successful pharmaceutical development. They are particularly important in cancer therapy where the therapeutic index of the drug is often quite small and the stability of biologic agents is limited.^{24, 40} *In situ*

*Corresponding author: Prof. Agata A Exner, Ph.D., Department of Radiology and Department of Biomedical Engineering, Case Western Reserve University, USA, 11100 Euclid Ave, Cleveland, OH, 44106-5056, Telephone: 1-216-744-3544, agata.exner@case.edu.

forming implants (ISFI) used for local drug delivery directly into the tissue of interest (e.g. a tumor) are an attractive alternative route to oral and intravenous administration.³⁶ Through a minimally invasive injection procedure using a small-gauge needle, ISFI can deliver effectively high dosages of drugs directly to the target site, minimize systemic side effects, and help increase the patient compliance through decreased frequency of administration.² PLGA, poly(lactic acid-co-glycolic acid) polymers have been used as a foundation for many ISFI formulations due to their biocompatibility and biodegradability, and their widely accepted use in drug delivery systems and other applications which have garnered approval of the Food and Drug Administration (FDA).²

Injectable implants using PLGA were developed over 25 years ago, but have not gained broad clinical acceptance.² This is especially true for applications where the desired site of action is localized in the immediate vicinity of the implants, such as in the case of intratumoral chemotherapy. In contrast, in applications where long term systemic drug activity is desirable, ISFI have been utilized more frequently.²⁵ Limited use of ISFIs in site-specific applications in cancer can be attributed to numerous factors, but one significant issue is the inadequate distribution of the drug locally upon release from the implants.³⁸ Prior studies report penetration of released doxorubicin in liver tissue in range of 2-3 mm from the implant boundary.³⁷ Distribution of SN-38 released from SN-38-loaded depots was 6 mm from the boundary after 20 days post-implantation in the brain.¹⁸ To address this challenge with ISFI, here we demonstrate a unique approach to increase local penetration of drug released locally from ISFI using ultrasound.

Ultrasound is a common diagnostic tool. In addition to common clinical applications ultrasound has also been used to facilitate drug release on demand in transdermal drug delivery systems and nanoparticles²⁰ and has been shown to improve the penetration of nanoscale particles at the target site, allowing them to pass through epithelial cells or barriers.^{11, 32, 33} In this process, typically it is the mechanical and thermal effects which are associated with an increase in penetration, perfusion or release of the drug.^{6, 33} Recently ultrasound was also used to control release of insulin from a gel-like network system consisting of alginate-coated nanoparticles and chitosan-coated nanoparticles. During treatment with focused ultrasound, the network was deformed and promoted insulin release.⁴ Low-intensity focused ultrasound is also used in number of applications to destroy gas-filled microbubbles, which leads to sonoporation and improved drug delivery parameter.⁶ Building upon this body of research, our current work examines the feasibility of using ultrasound to enhance drug molecule transport from in situ forming implants and throughout the surrounding injection site, here tissue mimicking poly(acrylamide) gel phantoms.

Materials and Methods

Chemicals

Sodium fluorescein and N-methyl-2-pyrrolidinone (NMP) were obtained from Sigma-Aldrich (St. Louis, MO). Poly(DL-lactic-co-glycolic) acid (PLGA) with a carboxylic acid end group, 50:50 DLG 4.5A, inherent viscosity 0.4 - 0.5 dL/g) was obtained from Lakeshore Biomaterials (Birmingham, AL). Acrylamide, bis-acrylamide, ammonium persulfate (APS),

and N,N,N',N'-tetramethylethylenediamine (TEMED) were obtained from Fisher Scientific (Waltham, MA).

Phantom preparation and in vitro distribution study

A tissue-mimicking hydrogel phantom (2 kPa elastic modulus) was prepared by free-radical polymerization of acrylamide (6.45 % (w/v)) and bisacrylamide (0.095 % (w/v)) using TEMED and APS.⁸ The phantoms (17 mL) were polymerized within a 6-well plate and kept in PBS solution for 5 days before implantation. The mixture solution of an implant was described in the previous work.^{8, 34} Briefly, PLGA was dissolved in NMP solvent and incubated at 37°C for 1 hour until PLGA was completely dissolved. Fluorescein was then added into the mixture solution and left in the incubator with circulation mixture at 37 °C for another 1 day. Herein, the mass ratio of PLGA:NMP:fluorescein was fixed at 39:60:1. Implants (~45 µL) were directly injected through 21G syringe needle into phantoms. To prevent the drug release through the needle track, the needle was held in place by a clamp at the injection site for 5 minutes before removal. Phantoms including implants were then kept in 60 mL of PBS, and incubated at 37°C with an orbital shaking at 80 rpm. A commercially available physical therapy ultrasound unit (Omnisound® 3000, Accelerated Care Plus Corp., Reno, NV) equipped with two collimating transducers was used to exposure ultrasound wave to samples. 3 hours after the implant was injected into the phantom, ultrasound was applied at either 0.7 W/cm² or 2.2 W/cm² intensity for 10 minutes (33% duty cycle, pulse duration 2 ms, 3 MHz). The application of ultrasound was repeated daily for the duration of the study. The setup is shown in Figure 1. To study effect of hyperthermia, phantoms with the same implants were incubated in a water bath at 42°C for 10 minutes in lieu of ultrasound application. To measure temperature, a thermocouple probe (HH-21A, OMEGA®) was inserted into the phantom proximal to the implant. The constructs were evaluated daily using a fluorescence imaging system (CRI Maestro, Caliper Life Science) with a blue excitation/emission filter (435-480 nm/515 nm) and an exposure time of 10 ms. Fluorescence intensity was analyzed using ImageJ (National Institutes of Health) with plugin “Radial Profile”.

Preparation of implants and measurement of in vitro release

To investigate *in vitro* release in PBS, the mixture (~45 µL) was injected into 30 ml of PBS to form an implant and placed at 37°C and 80 rpm. Ultrasound at 2.2 W/cm² with the same parameters as above was applied daily to each implant for 10 minutes. The distance between the transducer and implant was fixed at 1.5 cm. Implants were localized using a cap of a 15 mL centrifuge tube to reduce implant movement away from the sonication site during application of the ultrasound treatment. The same volume of PBS was replaced after ultrasound application. At the predetermined time, the amount of released fluorescein was determined via microplate reader (Tecan Ltd, Infinite 200 series) at an excitation wavelength of 485 nm and an emission wavelength of 525 nm.

In vitro release was also measured in the phantoms. Here, fluorescein-loaded implants (~45 µL) were injected into phantoms as for the distribution experiments. At predetermined time points (6 hours, 1, 3, 5, 7, 10, and 14 days), implants were removed from the phantoms and dissolved in 2 M NaOH to measure the amount of fluorescein that was remaining in the

removed implants. The amount of released fluorescein was calculated by comparing the residual fluorescein remaining in each implant to the initial loading amount at $t = 0$.

Degradation study

Implants were prepared as above, injected either into phantoms or into PBS and treated as described above using 2.2 W/cm^2 and the 4.0 cm^2 surface area probe. Implants were collected after 3, 7, and 14 days post-injection, washed with distilled water and freeze-dried. Implants were then dissolved in tetrahydrofuran (THF) to a concentration of 10 mg/ml followed by filtration ($0.2 \mu\text{m}$ PTFE syringe filter). Molecular weight of the polymers was analyzed by GPC (EcoSEC GPC system with RI and UV detector: wavelength at 254 nm) and TSKgel SuperHM-M column ($4.6 \text{ mm} \times 15 \text{ cm}$, $4 \mu\text{m}$) at a flow rate of 0.35 ml/min , 40°C .

Statistical analysis

All data were expressed as mean $\pm$ SD. All studies were carried out at least in triplicate. Significant differences within data were calculated and evaluated using unpaired t -test. P -value (< 0.05) was considered as statistically significant.

Results

Fluorescein distribution in phantoms

The distribution of fluorescein in poly(acrylamide) phantoms is shown in Figure 2. The overall implant mass of control group, water bath group, and treated groups at 0.7 , 2.2 W/cm^2 were 48.8 ± 5.4 , 47.3 ± 3.0 , 52.9 ± 2.2 , and 49.1 ± 1.3 , respectively. Upon qualitative examination, it can be seen that fluorescein distribution in implants that received treatment at 2.2 W/cm^2 was greater than the no-ultrasound control during day 2 to day 10 after implantation. Treatment with 0.7 W/cm^2 and in the 42°C water bath also did not appear to have any effect on the fluorescein distribution. In addition, only minor differences were noted between all groups during the first 2 days after injection (Supplementary Figure S1). Likewise, distribution of free unencapsulated fluorescein solution was not affected by ultrasound application. This experiment was carried out by injection of fluorescein solution (NMP) into the phantom and monitored by the same procedures as implants for 3 days (Supplementary Figure S2).

The mean radial fluorescence intensity profiles plotted from the center of each implant are shown in Figure 3. Signal intensity in the group treated with 2.2 W/cm^2 ultrasound was significantly higher than control during 3-10 days (Figure 3B – 3E) post-implantation. At the implant-phantom boundary ($\sim 0.2 \text{ cm}$ from center of implants), fluorescein intensities from treated group at 3, 5, 7, and 10 days post-implantation were 2.4, 6.0, 2.0, and 1.7-fold greater ($p < 0.05$) than control group, respectively, whereas group treated with 0.7 W/cm^2 had the same distribution as control. Likewise, at a distance of 1 cm from implant center, the intensities were 1.7, 5.6, 3.0, and 1.8-fold greater than control at 3, 5, 7, and 10 days post-implantation. Area under the curve (AUC) calculations are shown in Figure 4. The AUC of the implants receiving 2.2 W/cm^2 was significantly ($p < 0.05$) different at 3, 5, 7, and 10 days post-implantation from both the control group receiving no ultrasound and the group

heated in the 42°C water bath. In addition, change in temperature after ultrasound treatment was recorded to be near 5°C, Supplementary Figure S3. The temperature inside the phantoms from ultrasound treated group and the phantoms that were incubated in 42°C water bath increased at the same rate. Importantly a 10 min, 5 degree temperature change is not expected to have a major effect on normal tissue if this method were applied clinically.³⁹

Fluorescein release

In PBS, injected implants had a mass of 41.0 ± 0.6 mg and 42.6 ± 1.9 mg for control and ultrasound-treated groups, respectively. Average cumulative release of fluorescein was shown in Figure 5A. The initial burst was approximately 41% in the first 24 hours in the control group compared to 50% in the ultrasound-treated group. However, no significant differences were observed including with application of the therapeutic ultrasound. Implants injected into the phantoms had a mass of 47.8 ± 11.1 and 51.0 ± 6.2 mg for control and ultrasound-treated groups, respectively. Using the same criteria release kinetics^{8, 28, 35}, the ultrasound-treated group showed an increase in release during the diffusion phase (> 72 h) compared with the control, Figure 5B. This is consistent with the increase in drug distribution on days 3, 5, 7 and 10.

Polymer degradation

The molecular weight of PLGA of both control and treatment groups was found to be approximately $56.4 \pm 5.2\%$, $48.4 \pm 4.5\%$, and $22.3 \pm 1.2\%$ of the original value at 3, 7, and 14 days post-injection in PBS, Figure 6. In the poly(acrylamide) phantom, a slight but significant difference at 7 days post-injection was seen between the treated and control groups (23.7 ± 1.2 vs $28.4 \pm 2.4\%$). PLGA implants undergo bulk erosion.⁴¹ The implants upon contact with water undergo rapid ester bond cleavage throughout the system. Increase of acidic byproducts especially in the phantoms likely resulted in the rapid MW loss as shown in Figure 6. This degradation is much higher than that of PLGA implants without phantoms where the PBS was frequently replaced. The local pH of the implants in the phantoms was lower (data not shown) and generated more autocatalysis degradation rate. The GPC chromatograms of all implants were presented in Supplementary Figure S4.

Discussion

The use of polymeric implants for drug delivery has been extensively investigated with non-degradable polymers such as ethylene-vinyl alcohol and biodegradable polymer like PLGA.^{21, 36, 18, 36} However, regardless of the polymer matrix, overall local delivery of drugs or compounds has been limited by passive diffusion and low penetration and distribution. To overcome these challenges, ultrasound can be enlisted as a safe external force to facilitate drug release and distribution *in vivo*.^{11, 16, 32, 33} Several prior studies have examined the use of ultrasound to enhance drug release from polymer implants.^{5, 6, 22} In contrast to the current study, these studies included gas bubbles or generation of heat, which were able to drive drug release via inertial cavitation and/or mild hyperthermia. Ultrasound exposure has also been shown to significantly increase degradation rate of the polymer matrix, especially polyanhydrides, or reform the structure of the polymer such as cross-link hydrogel or shape memory polymers.^{4, 9, 12-14, 21} However, while the use of ultrasound for active, on demand,

delivery of drugs has been under extensive investigation^{1, 11, 30, 33}, its application in increasing drug distribution upon release from *in situ* forming polymer implants has not been investigated.

Compared to other techniques such as magnetic nanoparticles⁷, ultrasound is less costly, simpler and does not depend on a high local concentration of nanoparticles to achieve the desired effect. Different carriers and strategies capitalizing on the mechanical forces of ultrasound for drug delivery to tumors have been studied. These strategies are either applied to various existing drug loaded nanoparticles such as liposomes^{10, 29} or use ultrasound-visible microbubbles in combination with therapeutics.^{19, 31} Applying therapeutic ultrasound to *in situ* forming implants could become an alternative method to enhance release and distribution of agents without any additional gas bubbles. Herein, fluorescein-loaded PLGA implants were prepared in 2 kPa elastic modulus phantoms. This phantom had constant mechanical property for 14 days. The hydrogel phantom provides a physical structure with high water content and tunable biologically-relevant mechanical properties.^{8,27}

In this study, we investigated the application of ultrasound from a single transducer applied at a low (0.7 W/m²) and high (2.2 W/cm²) intensity on ISFIs and compared them to untreated controls. Overall, only the higher intensity ultrasound was found to significantly increase the rate of fluorescein release, polymer degradation and to effectively and significantly increase the drug penetration distance through hydrogel phantoms. In contrast, ultrasound application to implants formed in PBS had no perceived effect when the distance between the transducer and the implant was fixed at around 1.5 cm. Likewise, ultrasound applied to fluorescein solution (without entrapment in a polymer implant) had no effect on distribution at either intensity.

Overall, the total amount of fluorescein released in the phantoms was greater for implants treated with ultrasound. This effect was noted as early as 1 day after injection, and the increase was significant at 7 and 10 days. Increase in fluorescein release was not observed in PBS. Especially between 1 to 9 days post-implantation, fluorescein release in PBS (which was in the diffusion phase) was found to be 1-2 % per day. In contrast, for PLGA implants in the degradation phase (9 days post-implantation), the rate of fluorescein release was higher, which is consistent with prior reports.³⁵

A slightly faster degradation rate was noted for the ultrasound group at 3, 7 and 14 days in phantoms. The difference was significant after day 7 but no longer at day 14. Since the ester bond of PLGA was cleaved by water, the accumulation of lactic acid products could increase in the local pH (autocatalysis). For implants in the phantoms, a localized accumulation of the acidic byproducts could lead to increased PLGA degradation.⁴¹ It should be noted that implant from both groups were almost completely degraded after 21 days (data not shown). Overall, however, the differences were minor and thus are not thought to have contributed to the sustained increase in drug penetration.

When applied in the context of nanoparticles and/or bubbles, the mechanism of US-enhanced delivery is likely to be a combination of three factors: cavitation, acoustic streaming and hyperthermia, all resulting from interactions of particles and tissues with the

ultrasound waves.^{17, 33} These factors are used to increase permeability of vessels, cell walls, and the blood brain barrier, and can also be used to drive drug release from particles. The acoustic radiation force resulting from the longitudinal pressure waves moving through tissue can also be used to move particles along the same direction. This has been used to enhance particle interaction with targets, and alter the particle distribution within target tissue.^{17, 32}

The same acoustic radiation force is also the foundation of shear wave generation used for elastography imaging.^{3, 23} Shear wave elastography is used clinically to measure tissue stiffness in liver fibrosis and cancer.^{3, 15, 26} When examining our data, since hyperthermia had no effect on either drug release or distribution as shown in Figures 2-4, it is possible that, combined with increased diffusion, complementary mechanical effects propagating through the viscoelastic phantoms lead to the observed increase in the volume of distribution. It is important to note, however, that the hyperthermia via therapeutic ultrasound likely radiated from the implant outward, while that from the water bath radiated from the outside toward the implant. This could contribute to distinct effects on the implant itself and drug distribution within the implants. In addition, while hyperthermia may not be the driving force in this system, in future clinical applications in cancer therapy, the mild hyperthermia could certainly lead to synergistic therapeutic effects when combined with the local chemotherapy delivery.

As an alternative or additional mechanism for the observed effects, we hypothesize that it could be the implant itself, and its progressive hardening (resulting from solvent exchange and polymer precipitation) that acts as a seed for generation of these effects. Full implant solidification has been shown to take up to 3 days³⁴, and therefore may explain why the enhancement in drug penetration was not observed at the initial time points. It is also important to note that the release of fluorescein from the implants increased with the application of the higher intensity ultrasound. Additional in depth experiments are necessary to decouple these effects and establish the specific underlying mechanism driving these observations.

This study still has several limitations. Foremost are the limited acoustic parameters which could be manipulated with the available ultrasound equipment. In depth parameter optimization with a more robust acoustic setup and focused transducers could yield even greater enhancement and lead to understanding of the underlying mechanisms. Another limitation was the use of fluorescein as a mock drug. It should be noted that the previous study described the similarity between fluorescein and doxorubicin in terms of release profile, release kinetics in both *in vitro* and *in vivo*.³⁵ However, because implant behavior differs depending on the formulation, drug loading extent, polymer molecular weight and the like, additional research is required to understand the contribution of each of these on the observed acoustic-enhancement effect. Accordingly, the experiments here delineate only relative differences between the treatment groups and they cannot be used to accurately predict *in vivo* behavior. Additional experiments must be conducted to establish this strategy *in vivo* in an animal model, where the complexities of the tumor environment will play a significant role on the outcome.

In conclusion, the study demonstrates that a straightforward ultrasound protocol can be used to increase the release rate and spatial distribution of fluorescein released from *in situ* forming PLGA implants. Although the mechanism behind these observations is not entirely understood, we pose that coupled with increased local drug release, the acoustic radiation force interacting with the PLGA implants could result in increased drug release and penetration in the viscoelastic phantoms. This strategy could be eventually utilized to increase the treatment volume of ISFIs in tumors and lead to improved outcomes in treatment of various malignancies where local tumor control is desired.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to thank Dr. Michael Kolios for the fruitful scientific discussions. This research project was supported by National Institute of Biomedical Imaging and Bioengineering and National Cancer Institute of the national Institutes of Health under award numbers R01EB016960 (AAE and SJ), T32EB007509 (CH), and F31CA200373 (CH). The first author (CM) was supported by the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0126/2554). The third author (PN) was supported by the Royal Thai Government.

References

1. Boissenot T, Bordat A, Fattal E, Tsapis N. Ultrasound-triggered drug delivery for cancer treatment using drug delivery systems: From theoretical considerations to practical applications. *J Control Release*. 2016; 241:144–163. [PubMed: 27667179]
2. De Souza R, Zahedi P, Allen CJ, Piquette-Miller M. Polymeric drug delivery systems for localized cancer chemotherapy. *Drug Deliv*. 2010; 17:365–375. [PubMed: 20429844]
3. Deng Y, Rouze NC, Palmeri ML, Nightingale KR. Ultrasonic Shear Wave Elasticity Imaging (SWEI) Sequencing and Data Processing Using a Verasonics Research Scanner. *IEEE Trans Ultrason Ferroelectr Freq Control*. 2017; 64:164–176.
4. Di J, Price J, Gu X, Jiang X, Jing Y, Gu Z. Ultrasound-triggered regulation of blood glucose levels using injectable nano-network. *Adv Healthc Mater*. 2014; 3:811–816.
5. Epstein-Barash H, Orbey G, Polat B, Ewoldt R, Feshitan J, Langer R, Borden M, Kohane D. A microcomposite hydrogel for repeated on-demand ultrasound-triggered drug delivery. *Biomaterials*. 2010; 31:5208–5217. [PubMed: 20347484]
6. Gong Y, Wang Z, Dong G, Sun Y, Wang X, Rong Y, Li M, Wang D, Ran H. Low-intensity focused ultrasound mediated localized drug delivery for liver tumors in rabbits. *Drug Deliv*. 2016; 23:2280–2289. [PubMed: 25367869]
7. Gröll H, Langereis S. Hyperthermia-triggered drug delivery from temperature-sensitive liposomes using MRI-guided high intensity focused ultrasound. *J Control Release*. 2012; 161:317–327. [PubMed: 22565055]
8. Hernandez C, Gawlik N, Goss M, Zhou H, Jeganathan S, Gilbert D, Exner AA. Macroporous acrylamide phantoms improve prediction of *in vivo* performance of *in situ* forming implants. *J Control Release*. 2016; 243:225–231. [PubMed: 27742445]
9. Huebsch N, Kearney C, Zhao X, Kim J, Cezar C, Suo Z, Mooney D. Ultrasound-triggered disruption and self-healing of reversibly cross-linked hydrogels for drug delivery and enhanced chemotherapy. *Proc Natl Acad Sci USA*. 2014; 111:9726–9767.
10. Kheirrolomoom A, Lai CY, Even LM, Ingham ES, Fite BZ, Watson KD, Ferrara KW. Achieving complete response to locoregional disease without toxicity using temperature-sensitive liposomes and ultrasound-mediated hyperthermia. *Clin Cancer Res*. 2013:2155.

11. Klibanov AL, Hossack JA. Ultrasound in radiology: from anatomic, functional, molecular imaging to drug delivery and image-guided therapy. *Invest Radiol.* 2015; 50:657–670. [PubMed: 26200224]
12. Kost J, Leong K, Langer R. Ultrasound-enhanced polymer degradation and release of incorporated substances. *Proc Natl Acad Sci USA.* 1989; 86:7663–7666. [PubMed: 2813349]
13. Leong K, Kost J, Mathiowitz E, Langer R. Polyanhydrides for controlled release of bioactive agents. *Biomaterials.* 1986; 7:364–371. [PubMed: 3778996]
14. Li G, Fei G, Xia H, Han J, Zhao Y. Spatial and temporal control of shape memory polymers and simultaneous drug release using high intensity focused ultrasound. *J Mater Chem.* 2012; 22:7692–7696.
15. Liu Y, Fite BZ, Mahakian LM, Johnson SM, Larrat B, Dumont E, Ferrara KW. Concurrent visualization of acoustic radiation force displacement and shear wave propagation with 7T MRI. *PLoS One.* 2015; 10:e0139667. [PubMed: 26439259]
16. Liu Y, Paliwal S, Bankiewicz K, Bringas J, Heart G, Mitragotri S, Prausnitz M. Ultrasound-enhanced drug transport and distribution in the brain. *AAPS Pharm Sci Tech.* 2010; 11:1005–1017.
17. Lum AF, Borden MA, Dayton PA, Kruse DE, Simon SI, Ferrara KW. Ultrasound radiation force enables targeted deposition of model drug carriers loaded on microbubbles. *J Control Release.* 2006; 111:128–134. [PubMed: 16380187]
18. Manaspon C, Nasongkla N, Chaimongkolnukul K, Nittayacharn P, Vejjasilpa K, Kengkoom K, Boongird A, Hongeng S. Injectable SN-38-loaded polymeric depots for cancer chemotherapy of glioblastoma multiforme. *Pharm Res.* 2016; 33:2891–2903. [PubMed: 27495180]
19. McDannold N, Arvanitis CD, Vykhodtseva N, Livingstone MS. Temporary disruption of the blood–brain barrier by use of ultrasound and microbubbles: safety and efficacy evaluation in rhesus macaques. *Cancer Res.* 2012; 72:3652–3663. [PubMed: 22552291]
20. Mitragotri S. Healing sound: the use of ultrasound in drug delivery and other therapeutic applications. *Nat Rev Drug Discov.* 2005; 4:255–260. [PubMed: 15738980]
21. Miyazaki S, Yokouchi C, Takada M. External control of drug release: controlled release of insulin from a hydrophilic polymer implant by ultrasound irradiation in diabetic rats. *J Pharm Pharmacol.* 1988; 40:716–717. [PubMed: 2907539]
22. Moncion A, Arlotta K, O'Neill E, Lin M, Mohr L, Franceschi R, Kripfgans O, Putnam A, Fabiili M. *In vitro* and *in vivo* assessment of controlled release and degradation of acoustically responsive scaffolds. *Acta Biomater.* 2016; 46:221–233. [PubMed: 27686040]
23. Nightingale K, Soo MS, Nightingale R, Trahey G. Acoustic radiation force impulse imaging: in vivo demonstration of clinical feasibility. *Ultrasound Med Biol.* 2002; 28:227–235. [PubMed: 11937286]
24. Osswald CR, Kang-Mieler JJ. Controlled and extended release of a model protein from a microsphere-hydrogel drug delivery system. *ANN Biomed Eng.* 2015; 43:2609–2617. [PubMed: 25835212]
25. Owen A, Rannard S. Strengths, weaknesses, opportunities and challenges for long acting injectable therapies: Insights for applications in HIV therapy. *Adv Drug Deliv Rev.* 2016; 103:144–156. [PubMed: 26916628]
26. Palmeri ML, Wang MH, Dahl JJ, Frinkley KD, Nightingale KR. Quantifying hepatic shear modulus *in vivo* using acoustic radiation force. *Ultrasound Med Biol.* 2008; 34:546–558. [PubMed: 18222031]
27. Park SK, Guntur SRAR, Lee KI, Paeng DG, Choi MJ. Reusable ultrasonic tissue mimicking hydrogels containing nonionic surface-active agents for visualizing thermal lesions. *IEEE Trans Biomed Eng.* 2010; 57:194–202. [PubMed: 19770089]
28. Patel RB, Carlson AN, Solorio L, Exner AA. Characterization of formulation parameters affecting low molecular weight drug release from *in situ* forming drug delivery systems. *J Biomed Mater Res A.* 2010; 94:476–484. [PubMed: 20186771]
29. Pruitt JD, Pitt WG. Sequestration and ultrasound-induced release of doxorubicin from stabilized pluronic P105 micelles. *Drug Deliv.* 2002; 9:253–258. [PubMed: 12511204]

30. Rapoport, N. Drug-Loaded Perfluorocarbon Nanodroplets for Ultrasound-Mediated Drug Delivery. In: Escoffre, JM., Bouakaz, A., editors. Therapeutic Ultrasound. Cham: Springer International Publishing; 2016. p. 221-241.
31. Rychak JJ, Klibanov AL. Nucleic acid delivery with microbubbles and ultrasound. *Adv Drug Deliv Rev.* 2014; 72:82–93. [PubMed: 24486388]
32. Shortencarrier MJ, Dayton PA, Bloch SH, Schumann PA, Matsunaga TO, Ferrara KW. A method for radiation-force localized drug delivery using gas-filled lipospheres. *IEEE Trans Ultrason Ferroelectr Freq Control.* 2004; 51:822–831. [PubMed: 15301001]
33. Sirsi SR, Borden MA. State-of-the-art materials for ultrasound-triggered drug delivery. *Adv Drug Deliv Rev.* 2014; 72:3–14. [PubMed: 24389162]
34. Solorio L, Exner AA. Effect of the subcutaneous environment on Phase-sensitive *in situ*-forming implant drug release, degradation and microstructure. *J Pharm Sci.* 2015; 104:4322–4328. [PubMed: 26506522]
35. Solorio L, Olear AM, Zhou H, Beiwenger AC, Exner AA. Effect of cargo properties on *in situ* forming implant behavior determined by noninvasive ultrasound imaging. *Drug Deliv Transl Re.* 2012; 2:45–55.
36. Solorio L, Wu H, Hernandez C, Gangolli M, Exner AA. Ultrasound-guided intratumoral delivery of doxorubicin from *in situ* forming implants in a hepatocellular carcinoma model. *Ther Deliv.* 2016; 7:201–212. [PubMed: 27010983]
37. Weinberg BD, Ai H, Blanco E, Anderson JM, Gao J. Antitumor efficacy and local distribution of doxorubicin via intratumoral delivery from polymer millirods. *J Biomed Mater Res A.* 2007; 81:161–170. [PubMed: 17120197]
38. Weiser JR, Saltzman WM. Controlled release for local delivery of drugs: barriers and models. *J Control Release.* 2014; 190:664–673. [PubMed: 24801251]
39. Yarmolenko PS, Moon EJ, Landon C, Manzoor A, Hochman DW, Viglianti BL, Dewhirst MW. Thresholds for thermal damage to normal tissues: An update. *Int J Hyperthermia.* 2011; 27:320–343. [PubMed: 21591897]
40. Yavuz B, Pehlivan SB, Kaffashi A, Çalamak S, Ulubayram K, Palaska E, Çakmak HB, Ünlü N. *In vivo* tissue distribution and efficacy studies for cyclosporin A loaded nano-decorated subconjunctival implants. *Drug Deliv.* 2016; 23:3279–3284. [PubMed: 27027148]
41. HB, Yelles MC., Tan, VT., Danede, F., Willart, JF., Siepmann, J. PLGA implants: How poloxamer/PEO addition slows down or accelerates polymer degradation and drug release. *J Control Release.* 2017; 253:19–29. [PubMed: 28284831]

Figure 1.

Schematic of enhancement of fluorescein distribution via therapeutic ultrasound using poly(acrylamide) gel phantom as a mimicking tissue. The therapeutic ultrasound was daily exposed from the top of the phantom for 10 minutes at 3 MHz and 33 % duty factor.

Figure 2.

Effect of ultrasound on fluorescein distribution in poly(acrylamide) gel phantoms. The phantoms were randomly classified into 3 groups including control, incubating in water bath, and ultrasound treated group. For incubating in water bath group, phantoms were daily incubated in water bath at 42°C for 10 minutes. Treated samples were treated with daily therapeutic ultrasound for 10 minutes at either 0.7 or 2.2 W/cm², 3 MHz, and 33 % duty factor.

Figure 3. Distribution profiles of fluorescein in poly(acrylamide) phantoms after implantation for 6 hours (A), 3 days (B), 5 days (C), 7 days (D), 10 days (E), and 14 days (F). For the 42°C group, phantoms were incubated in 42°C water bath for 10 minutes. Treated samples were treated either with therapeutic ultrasound for 10 minutes at either 0.7 or 2.2 W/cm², 3 MHz, and 33 % duty factor. The dash black line represented the boundary of implants. Each value represents the mean $\pm$ SD (control group, $n = 6$; other groups, $n = 3$). * designates significant differences from the control group ($p < 0.05$).

Figure 4.

Area under the curve (AUC) calculated from distribution profiles (mean $\pm$ SD). For the water bath group (42°C), phantoms were incubated in a water bath for 10 minutes. Treated samples were treated either with therapeutic ultrasound for 10 minutes at either 0.7 or 2.2 W/cm², 3 MHz, and 33 % duty factor. Each value represents the mean $\pm$ SD (control group, $n = 6$; other groups, $n = 3$). * designates significant differences from the control group ($p < 0.05$).

Figure 5.

Cumulative release of fluorescein in PBS (A) and standard phantom (B) with and without daily therapeutic ultrasound for 10 minutes (2.2 W/cm^2 , 3 MHz, and 33 % duty factor). Each value represents the mean $\pm$ SD ($n = 3$). * designates significant differences from the control group ($p < 0.05$).

Figure 6.

Degradation evaluated as loss in the molecular weight of 4.5A PLGA in PBS, pH = 7.4 (A) and phantom (B) with and without daily therapeutic ultrasound for 10 minutes (2.2 W/cm², 3 MHz, and 33 % duty factor). Each value represents the mean $\pm$ SD ($n = 3$). * designates significant differences from the control group ($p < 0.05$).