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The role of cavitation in acoustically activated drug delivery

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Abstract

Pluronic P105 micelles are potential candidates as chemotherapy drug delivery vehicles using ultrasonic stimulation as a release trigger. Acoustic power has been previously shown to release two anthracycline agents from these polymeric carriers. In this study, an ultrasonic exposure chamber with fluorescence detection was used to examine the mechanism of doxorubicin release from P105 micelles. Acoustic spectra were collected and analyzed, at the same spatial position as fluorescence data, to probe the role of cavitation in drug release. Our study showed a strong correlation between percent drug release and subharmonic acoustic emissions, and we attribute the drug release to collapse cavitation that perturbs the structure of the micelle and releases drug.

Keywords: Doxorubicin; Pluronic micelles; Fluorescence measurements; Cavitation events; Acoustic spectroscopy

1. Introduction

The triblock copolymer Pluronic P105 (PEO_{37}–PPO_{56}–PEO_{37}) has been found to be an ideal ultrasonically activated drug delivery vehicle for various reasons. First, the copolymer forms dense micelles with strongly hydrophobic cores at concentrations of 4 wt.% or above [1]. Second, the polypropylene oxide core is sufficiently hydrophobic to sequester hydrophobic drugs. Third, and perhaps most importantly, these micelles have been shown to release their contents upon the application of low frequency ultrasound, suggesting that drug molecules can be delivered specifically to the insonated region [2]. Fourth, when ultrasound is turned off the drug is quickly re-encapsulated inside the core of the micelles, thus possibly preventing the spread of drug outside the targeted region [3]. Fifth, polyethylene glycol chains prevent the carrier from being cleared by cells of the reticuloendothelial system, thus increasing the circulation time of the encapsulated drug. Sixth, Pluronic micelles are large enough to escape renal excretion while being small enough to extravasate at the tumor site. Seventh, Pluronic has been shown to be non-toxic at low

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concentrations and it has been used to overcome multidrug resistance (MDR) [4].

Previously, we have used an ultrasonic exposure chamber with real-time fluorescence detection to measure the release of doxorubicin (Dox) from unstabilized as well as stabilized Pluronic P105 micelles using ultrasonic stimulation [3,5]. The amount of drug release increased with ultrasonic intensity and decreased with frequency. Our previous studies confirmed the hypothesis that Dox was encapsulated in Pluronic P105 micelles and released upon exposure to ultrasound. Such ultrasonically controlled release has been effective against cancer cells in vitro [6] and in vivo [7,8].

In the research presented in this paper, we examine more thoroughly the mechanism of Dox release from Pluronic P105 micelles using a modified version of the ultrasonic exposure chamber used previously. The new design is more efficient at capturing the change in drug fluorescence within a particular volume upon the application of ultrasound, thereby allowing for a more accurate localized calculation of drug release. We concurrently use acoustic spectroscopy to examine the role of cavitation in releasing Dox from these micelles. The aim is to measure the acoustic intensity and acquire acoustic frequency spectra at the same location where the change in fluorescence is measured, which allows us to correlate release data to acoustic cavitation events caused by ultrasound.

Cavitation is the oscillation of gas bubbles upon exposure to pressure waves [9]. Cavitation is generally divided into two types of behavior. Stable cavitation is the repeatable oscillation of bubble diameter without leading to bubble collapse, and it occurs at relatively lower acoustic intensities. At higher intensities, the bubble oscillates sufficiently that the inertia of the inward moving water causes the bubble to collapse violently, producing shock waves, high temperatures, and free radicals [10]. This type of cavitation is called inertial or collapse cavitation.

For a continuous driving acoustic frequency (f), the pressure waves generated by cavitating bubbles contain harmonic (nf, where n=1, 2, 3...), subharmonic (f/2), and ultraharmonic frequencies ((2n+1)f/2, where n=1, 2, 3...) [11–13]. Stable cavitation has been associated with the observation of the harmonic and subharmonic frequencies [9,11,14]. However, inertial cavitation as evidenced by sonoluminescence, generation of free radicals, and the intensity of the non-harmonic background noise also has been correlated with subharmonic acoustic emission [15–18]. A positive correlation between Dox release and harmonic or subharmonic acoustic emissions in the absence of non-harmonic background noise would show that stable (not inertial) cavitation causes drug release. Non-harmonic background is attributed to the broadband emission produced by the shock wave resulting from collapse cavitation. If the percent release corresponds to the amount of a shift in the baseline of the acoustic pressure spectrum, then drug release could be attributed to collapse cavitation. This paper presents our observed correlation of drug release with the subharmonic emissions and sufficient background shift to implicate collapse cavitation in the drug release.

2. Materials and methods

2.1. Drug encapsulation in Pluronic micelles

Doxorubicin (Dox) was obtained from the University of Utah Hospital (Salt Lake City, UT) in a 1:5 mixture with lactose; it was dissolved in phosphate buffered saline (PBS) and sterilized by filtration through a 0.2 Am filter.

Stock solutions of Pluronic P105 (BASF, Mount Olive, NJ) were prepared by dissolving P105 in a PBS solution to a final concentration of 10 wt.%. Dox was dissolved into the P105 solutions at room temperature to produce a final Dox concentration of 10 Ag/mL in 10 wt.% Pluronic. The same drug concentration was also prepared in PBS.

The fluorescence of Dox at concentrations ranging from 2.5 to 80 Ag/mL in PBS and in 10% Pluronic was measured in a luminescence spectrometer (LS50B, Perkin-Elmer) with an excitation wavelength of 488 nm and emission intensity measured at 590 nm. Although self-quenching occurred at higher concentrations, Dox fluorescence in P105 was always greater than that in PBS, and below 10 Ag/mL the

change in fluorescence was fairly proportional to the change in Dox concentration for both solutions.

2.2. Measuring ultrasound-triggered release of Dox from Pluronic P105 micelles

A modification of the previous apparatus [2] was used in these experiments. The 488 nm beam of an argon ion laser (Ion Laser Technology, 5500 A) was directed through a dielectric-interface beam splitter; the intensity of the split portion of the beam was measured using a photodetector (Newport 818-SL with 835 display) and used to monitor the laser power throughout our experiments. The other portion of the beam was focused by a 20 microscope objective into one branch of a bifurcated fiber optic bundle (# DF13036M, Edmunds Optics, Barrington, NJ) that directed the light into an acoustically transparent plastic tube (cellulose butyrate, Tulox Plastics, Marion, Indiana), with a diameter of 2.54 cm, filled with the Dox solution. The laser light exited the common end of the bifurcated fiber optic bundle in a 0.09 sr cone of light. Dox molecules within the cone of light absorb at 488 nm and isotropically emit fluorescent light within a spectrum of about 530 to 630 nm. In the same fiber optic bundle a portion of the fibers were used to collect and direct the fluorescence to a detector. Numerical integration of a mathematical model of isotropic fluorescence within the cone of excitation light showed that about 99% of the collected fluorescence originated from within 3 mm of the fiber optic tip. The collected fluorescence signal was directed through the second branch of the fiber optic bundle through a dielectric bandpass filter (Omega Optical 535DF35) to a silicon detector (EG and G 450-1). The filter was used to reject any emissions with a wavelength below about 517 nm, including any Rayleigh-scattered laser light. The photodetector signal was captured on an oscilloscope (Tektronix TDS 3012) and subsequently stored on a computer for further processing.

This apparatus can measure the amount of acoustically activated Dox release from micelles because Dox exhibits a decrease in fluorescence in contact with an aqueous solution. Such is the case when Dox is released from Pluronic micelles, and it follows that the magnitude of decrease in fluorescence intensity upon application of ultrasound provides a quantifiable measure of drug release.

The decrease in fluorescence of the encapsulated drug solution was assumed to be directly proportional to the amount of drug released relative to a known baseline. The fluorescence of Dox in PBS, in the absence of Pluronic, was measured to simulate 100% release. Then the percent release was calculated using the following:

$$\%\ \text{release} = \frac{I_{P105} - I_{US}}{I_{P105} - I_{PBS}} \times 100\% \qquad (1)$$

where I_{US} is the fluorescence intensity upon exposure to ultrasound, I_{PBS} is the fluorescence intensity in a solution of free Dox in PBS, and I_{P105} is the intensity recorded when the drug is encapsulated in Pluronic P105 (which corresponds to 0% release and 100% encapsulation).

In these experiments, the fluorescence intensity of the drug in PBS was measured both with and without the application of ultrasound. Ultrasound was applied using a 70-kHz ultrasonicating bath (SC-100, Sonicor, Copiaque, NY) equipped with two piezoceramic transducers that are driven at about 70 kHz. The best description of the waveform is that of a 70-kHz wave amplitude modulated sinusoidally at about 0.12 kHz. The bath was powered by 60-Hz AC voltage from a variable AC transformer (variac). The voltage from the variac to the sonicating bath was adjusted to produce differing intensities of ultrasound. To execute the experiments, the end of the fiber optic was positioned at an acoustically intense position in the ultrasonicating bath, as determined by a hydrophone (described below). The bath was filled with degassed water and the tube surrounding the fiber optic was filled with the solution of Dox in PBS. Fluorescence emissions were collected for different voltages applied to the ultrasonicating bath. Then, without changes in the experimental set-up, the Dox solution in PBS was carefully removed and replaced with a Dox solution of the same concentration in Pluronic micelles. During insonation, fluorescence dropped due to Dox coming in contact with the surrounding aqueous environment. Several

fluorescence measurements were made at each intensity setting and averaged (n=8 when IN0.27 W/cm^2 and n=4 when IV0.27 W/cm^2).

2.3. Acoustic measurements

Ultrasonic power density measurements were obtained using a calibrated hydrophone (Bruel and Kjaer 8103, Decatur, GA) whose output voltage was monitored with an oscilloscope. After measurements of Dox fluorescence, the fiber optic was replaced with the hydrophone in the same location, and the hydrophone response was recorded at the same settings as used for the fluorescence measurements. The average acoustic intensity was calculated using $I=V_{rms}^2Q^2/Z$ where Q is the frequency-dependent calibration factor obtained from the manufacturer that relates pressure to voltage, Z is the acoustic impedance of water (1.5 Mrayl), and V_{rms} is the rootmean-squared voltage of the hydrophone signal.

Acoustic spectroscopy was used to monitor the spectra of the vibrations of the cavitating bubbles in the ultrasonic field at the power settings used in the release measurements. The hydrophone signal was directed to a spectrum analyzer (Agilent E4401B), which displayed and recorded the acoustic spectrum.

3. Results

The experimental apparatus employed in this research enabled us to measure the change in fluorescence and to analyze the acoustic spectrum accompanying the change. Fig. 1 shows the percent of doxorubicin release (calculated using the change in fluorescence and Eq. (1)) from micelles as a function of the average power density delivered. No significant (pN0.05) change in fluorescent intensity is seen below approximately 0.28 W/cm^2. At this power density the change in fluorescence increases and then levels off at higher intensities.

The three insets in Fig. 1 show examples of the frequency spectra collected during the experiments. The spectrum on the lower left contains no subharmonic peak (in the vicinity of 35 kHz) and was collected at an intensity (0.25 W/cm^2) where no Dox release was detected. The inset on the lower right shows the development of a subharmonic peak (at approximately 35 kHz) at 0.28 W/cm^2. At the same power density, the first measurable change in fluorescence was observed. There was also a large increase in

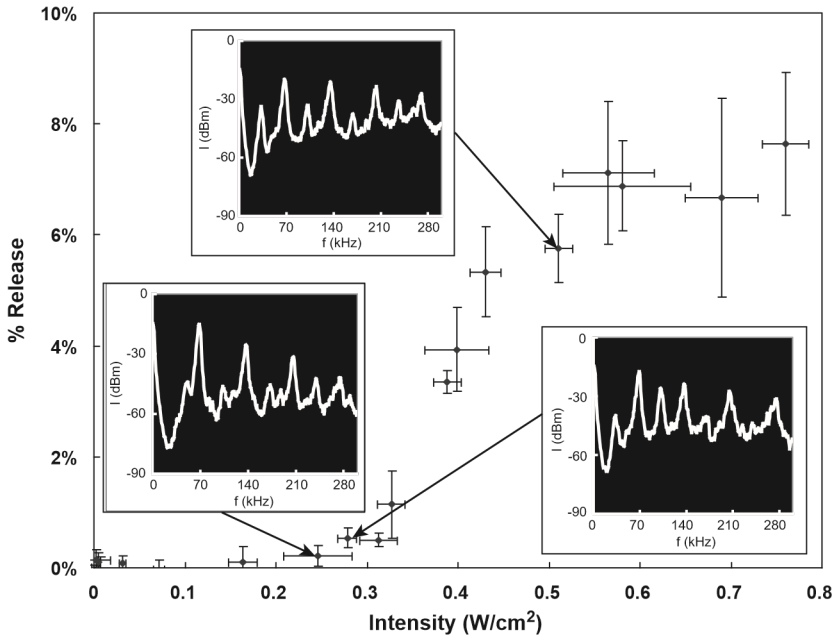

Fig. 1. Average percent release of doxorubicin calculated from a change in fluorescence intensity as a function of the ultrasonic intensity at 7 kHz. The error bars represent the standard deviations (n N4) of the mean. The three insets show the acoustic spectra at 0.25, 0.28, and 0.52 W/cm^2.

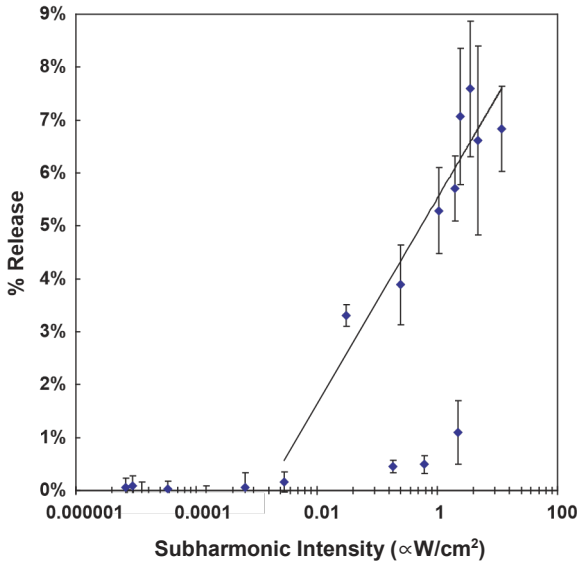

Fig. 2. Percent doxorubicin release from Pluronic micelles correlated with the acoustic intensity of the subharmonic peak. The error bars represent the standard deviations of the mean.

the level of the baseline when the intensity increased from 0.25 to 0.28 W/cm^2. The inset spectrum at the top shows that with increasing intensity, the subharmonic peak increased in magnitude, as did the magnitude of the baseline.

The onset of ultrasonically activated decrease in fluorescence (attributed to release of Dox) corresponds to the emergence of a subharmonic peak. Furthermore, this change in fluorescence increases as the magnitude of the subharmonic peak increases above a threshold value. Fig. 2 shows the acoustic intensity of the subharmonic signal correlated with the drug release (from fluorescence change). In general, as the subharmonic acoustic intensity increases, so does the change in fluorescence and, by extension, the drug release. There are some outliers to this trend, as will be discussed later.

4. Discussion

An ideal drug delivery system would be one in which the drug is sequestered inside a carrier until it can be released at the targeted point in space and time. We have previously shown that drug carriers formulated from micelles of Pluronic surfactant have the capacity to sequester hydrophobic drugs, and then release them upon the application of ultrasound. However, the mechanisms producing such controlled release were speculative and not well understood.

As with our previous publications, the results of the present experiment indicate that ultrasound releases a fluorescent drug from a Pluronic micelle [2]. However, these experiments go further toward revealing the mechanism of acoustically activated drug delivery [19,20]. The use of the bifurcated fiber optic emitter/collector bundle allowed us to measure the change in fluorescence in a localized volume; we then used the hydrophone to collect the acoustic spectrum at the same location under essentially identical experimental conditions.

4.1. Threshold for release

It is noteworthy that the amount of release is not a linear function of ultrasonic intensity (see Fig. 1). In particular, there is a threshold near 0.3 W/cm^2. This non-linear behavior was not apparent in previous experiments because insufficient data were collected near this threshold [21].

Observations of thresholds associated with cavitation and other ultrasonic phenomena are not unusual. The existence of a threshold for the onset of inertial or collapse cavitation is very well documented [9,15,22– 24]. Daniels et al. observed the threshold for inertial cavitation by trapping bubbles in agar gel. They found thresholds from 0.036 to 0.141 W/cm^2 at 750-kHz insonation [22]. Hill studied thresholds of inertial cavitation by iodine release and by monitoring the subharmonic signal. From 0.25 to 4 MHz the thresholds were near, and sometimes below, 1 W/cm^2, depending upon the frequency and whether the water was degassed [15]. Other authors have calculated the threshold for the onset of inertial cavitation as a function of frequency and bubble size [25,26].

There are numerous publications reporting ultrasonic intensity thresholds for an observed biological effect [15,27–36]. These thresholds are usually different than thresholds for collapse cavitation and vary based on the type of cells or tissue and the mechanism producing the effect

(permeabilizing cells, DNA delivery, lysis or tissue damage).

The group of Mitrogotri has convincingly shown that the thresholds for producing skin permeability are a strong function of ultrasonic frequency [13,31, 35,37,38]. As an example, the threshold for permeabilizing skin with 76.6 kHz is approximately 0.9 W/cm^2 [35].

Reports of thresholds for producing cell membrane damage or increased permeability are abundant [12,27,28,36,39]. Interestingly, some of these thresholds are in the region of stable cavitation, indicating that collapse cavitation is not required for cell membrane damage [39]. There is a reported threshold for DNA delivery to rabbit endothelial cells of about 2000 W/cm^2 at 0.85 MHz (pulse average intensity) [28]. This is obviously much higher than the threshold observed in this work.

Thresholds for lysing or causing major tissue damage differ depending on the tissue. For example, Rapoport et al. reported a threshold of 0.06 W/cm^2 at 20 kHz for HL-60 lysis [40].

4.2. Correlation with subharmonic

It is intriguing that the amount of release correlates fairly well with subharmonic intensity, again above a threshold value (see Fig. 2). There are three data points that lie outside this correlation, but they all occurred near the onset of drug release and onset of subharmonic emission (in the range from 0.27 to 0.34 W/cm^2 intensity). Even the subharmonic at 2.3 AW/cm^2 that is associated with a release of 1% was observed at 0.335 W/cm^2 total intensity. In these three boutlierQ observations, subharmonic signals appeared in the recorded acoustic spectra with very little corresponding measurement of drug release. Some reports have suggested that near the onset of subharmonic activity, the subharmonic emission is intermittent [11,41] and chaotic [9]; perhaps such an intermittent phenomenon in our system produced a short burst of subharmonic activity that was captured by the spectrum analyzer, but that did not persist temporally long enough to produce measurable drug release, which requires about 200 ms [3].

The subharmonic is an interesting enigma in cavitation, and the literature is not clear as to whether the subharmonic emission is produced by stable or by inertial cavitation. Some reports teach that some kinds of stable cavitation produce a subharmonic emission in the absence of any collapse cavitation [12–14,42]. It is notable that subharmonics associated with stable cavitation are produced at fairly high intensities, and could be near the onset of collapse cavitation.

With respect to the relationship between collapse cavitation and the subharmonic, there are reports that correlate subharmonic emission with positive indicators of collapse cavitation, such as iodine release, sonoluminescence and acoustic white noise [15–18]. Leighton maintains that cavitating bubbles produce a signal at f/2 because of a prolonged expansion phase immediately preceding a delayed collapse phase of the inertial cavitation event [11]. Thus there are varied opinions as to whether the presence of a subharmonic peak is a definitive indicator of collapse cavitation.

There is a reported correlation between biological phenomena and subharmonics, as Sundaram et al. have shown [13]. They found that (1) the viability and membrane permeability of 3T3 mouse cells increased as acoustic white noise in the frequency spectra increased, and (2) that cell viability and cell membrane permeabilization was independent of subharmonic energy density. The group concluded that ultrasound-induced permeabilization of cell membranes is caused by inertial, and not stable, cavitation events. On the other hand, Liu et al. have found a strong dependence in the degree of hemolysis (permeabilization of red blood cells as measured by the degree of hemoglobin release) on the subharmonic and ultraharmonic pressures, but not on the broadband or white noise [12]. That group concluded that the best correlation between ultrasonic parameters and hemolysis was the product of the total ultrasonic exposure time and the subharmonic pressure. Thus again there are varied opinions as to whether the subharmonic always correlates with biological phenomena.

In the present work, the most important question to address is what phenomena associated with subharmonic bubble vibrations could cause release from micelles. Oscillating bubbles, even in stable

cavitation, create very strong shear forces near the surface of the bubble. For example, the velocity of water near the surface of a 10 Am (diameter) hemispherical bubble, with a 1 Am oscillation amplitude and 70-kHz oscillation frequency is on the order of 1 m/s [43]. Even more noteworthy are the extremely high viscous shear rates near the surface of the bubble, which in this example are on the order of 10^5 s^1. This shear rate is equivalent to shearing water in a 1 mm gap between parallel plates in which one plate is stationary and the other moving at 100 m/s. These shear forces may be strong enough to shear open a loosely aggregated micelle of surfactant and expose its contents to the aqueous environment. However, we still have no direct theoretical or experimental evidence to relate such strong shear forces to the observation of a subharmonic bubble vibration.

The strong correlation of the subharmonic frequency with drug release from micelles is readily apparent from these data. Bubble cavitation dynamics produces this subharmonic emission; however, the relationship between cavitation and drug release is less apparent. As mentioned, subharmonic emission has been associated with both stablecavitation andcollapse cavitation, although association with the former is observed at moderate intensities just below the onset of collapse cavitation and under carefully controlled conditions where the bubble oscillations are non-linear, but not yet chaotic [14,41,42]. There are two other signatures of collapse cavitation that we can search for to establish whether stable or collapse cavitation is associated with the subharmonic, and thus is associated with the drug release. A definitive signature of collapse cavitation is the formation of free radicals, purported to arise from thermal decomposition of molecules under the extreme temperatures that are produced in a symmetric collapse of a bubble [10,44]. There are previous reports of the capture of free radicals by the spin trap 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) under experimental conditions nearly identical to those employed in this paper. In one case, radicals were trapped for intensities between 0.68 and 3.4 W/cm^2 [45], and in another case, radicals were trapped for intensities between 1.0 and 2.0 W/cm^2 [46]. Although the lower limits of the intensities are greater than the threshold observed herein for drug release, they are of the same order of magnitude. One cannot rule out the possibility that collapse cavitation occurred at lower levels but in lesser amounts such that it was difficult to trap a measurable number of radicals.

A second signature of collapse cavitation is the emission of a shock wave as the bubble implodes. Because the shock wave contains a wide spectrum of frequencies (white noise), this produces an increase in the baseline of the emission spectrum. Correlated with the appearance of the subharmonic was an increase in the background or baseline level of the acoustic spectrum. The insets in Fig. 1 show this jump, which is fairly large, considering that the y-axis of the insets is in decibels, a logarithmic scale. We submit that this increase in background acoustic emission establishes the connection between collapse cavitation, the appearance of the subharmonic, and drug release from the micelles.

One other piece of supporting evidence comes from the parameter of bmechanical indexQ, defined as the ratio of the peak negative pressure (in MPa) and the square root of the frequency (in MHz). This parameter was established by the American Institute of Ultrasound in Medicine (AIUM) as a measure of the likelihood of collapse cavitation occurring during ultrasonography [27]. The mechanical index is based on the work by Apfel and Holland who solved mathematical models of bubble dynamics to establish a threshold for the onset of collapse cavitation during pulsed insonation [26]. There are reports of bubble destruction (i.e., collapse cavitation) by pulsed ultrasound at a mechanical index as low as 0.4 [47]. Continuous insonation (as in our work) is expected to produce collapse cavitation at even lower values of the mechanical index [26]. In our work, the 0.28 W/cm^2 threshold for the appearance of the subharmonic corresponds to a mechanical index of 0.35. Again this supports our position that the appearance of the subharmonic peak is evidence of collapse cavitation.

Still the question remains as to how collapse cavitation could release drug form the interior of the micelle. Granted a micelle immediately adjacent to a collapsing bubble would be subject to very high shear stresses, perhaps sufficiently to shear apart the micelle and release the drug for a time until the micelle

reforms and re-encapsulates the drug. This might cause release from a very small fraction of micelles that reside within a few hundred nanometers of the bubble. The resulting shock wave, however, spreads into the fluid. The expanding shock wave consists of compressed and rarefied fluid, and creates high shear stresses as it passes through the liquid at the speed of sound. However, the intensity of the shock wave and the associated shear forces diminish as the wave expands. Recent studies have proposed a model for the interaction of the expanding shock wave (and its associated shear stresses) with cells suspended in the media [13,48]. By knowing the rupture strain of cell membranes, this model was used to predict a bblast radiusQ and the corresponding fraction of cells perturbed by the expanding shock wave. Unfortunately, we do not know the rupture stress or strain of our Pluronic micelles, and thus such a calculation of blast radius cannot be made. However, we argue that there is a distance (however unknown) from the bubble collapse event within which the shear stress would be sufficient to disrupt the structure of the micelle and release drug. Thus our qualitative model of drug release is that the expanding shock wave from a collapse cavitation event shears open micelles that are in sufficient proximity to the event.

In conclusion, our data confirm the connection between drug release from the micelles, and subharmonic acoustic emission. There is strong supportive evidence that collapse cavitation is involved with the drug release, most probably through high shear forces that disrupt the structure of the micelle. Obviously, more experiments must be conducted using ultrasonic transducers operating at different frequencies (above and below 70 kHz) to verify the coincidence of drug release, subharmonic emission and collapse cavitation at frequencies that may be more conducive to targeted ultrasonic drug delivery. Additionally, it is important to understand the conditions under which cavitation is generated in order to optimize the acoustic conditions to be used in ultrasonically activated drug delivery from polymeric micelles.

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References

[1] N. Rapoport, K. Caldwell, Structural transitions in micellar solutions of Pluronic P-105 and their effect on the conformation of dissolved Cytochrome C: an electron paramagnetic resonance investigation, Colloids Surf., B Biointerfaces 3 (1994) 217–228.

[2] G.A. Husseini, G.D. Myrup, W.G. Pitt, D.A. Christensen, N.Y. Rapoport, Factors affecting acoustically triggered release of drugs from polymeric micelles, J. Control. Release 69 (2000) 43–52.

[3] G.A. Husseini, N.Y. Rapoport, D.A. Christensen, J.D. Pruitt, W.G. Pitt, Kinetics of ultrasonic release of doxorubicin from Pluronic P105 micelles, Colloids Surf., B Biointerfaces 24 (2002) 253–264.

[4] A. Venne, S. Li, R. Mandeville, A. Kabanov, V. Alakhov, Hypersensitizing effect of Pluronic L61 on cytotoxic activity, transport, and subcellular distribution of doxorubicin in multiple drug-resistant cells, Cancer Res. 56 (1996) 3626–3629.

[5] G.A. Husseini, D.A. Christensen, N.Y. Rapoport, W.G. Pitt, Ultrasonic release of doxorubicin from Pluronic P105 micelles stabilized with an interpenetrating network of N,N-diethylacrylamide, J. Control. Release 83 (2) (2002) 302–304.

[6] G.A. Husseini, R.I. El-Fayoumi, K.L. O'Neill, N.Y. Rapoport, W.G. Pitt, DNA damage induced by micellar-delivered doxorubicin and ultrasound: comet assay study, Cancer Lett. 154 (2000) 211–216.

[7] J.L. Nelson, B.L. Roeder, J.C. Carmen, F. Roloff, W.G. Pitt, Ultrasonically activated chemotherapeutic drug delivery in a rat model, Cancer Res. 62 (2002) 7280–7283.

[8] Z.G. Gao, H.D. Fain, N. Rapoport, Controlled and targeted tumor chemotherapy by micellar-encapsulated drug and ultrasound, J. Control. Release 102 (1) (2005) 203–222.

[9] C.E. Brennen, Cavitation and Bubble Dynamics, Oxford University Press, New York, p. 282.

[10] K.S. Suslick, Sonochemistry, Science 247 (1990) 1439–1445.

[11] T.G. Leighton, The Acoustic Bubble, Academic Press, London, p. 613.

[12] J. Liu, T.N. Lewis, M.R. Prausnitz, Non-invasive assessment and control of ultrasound-mediated membrane permeabilization, Pharm. Res. 15 (6) (1998) 918–924.

[13] J. Sundaram, B.R. Mellein, S. Mitragotri, An experimental and theoretical analysis of ultrasound-induced permeabilization of cell membranes, Biophys. J. 84 (5) (2003) 3087–3101.

[14] J.L. Mestas, P. Lenz, D. Cathignol, Long-lasting stable cavitation, J. Acoust. Soc. Am. 113 (3) (2003) 1426–1430.

[15] C.R. Hill, Ultrasonic exposure thresholds for changes in cells and tissues, J. Acoust. Soc. Am. 52 (2) (1971) 667–672.
[16] T. Gudra, K.J. Opielinski, Applying spectrum analysis and cepstrum analysis to examine the cavitation threshold in water and in salt solution, Ultrasonics 42 (1–9) (2004) 621–627.
[17] G.K. Johri, D. Singh, M. Johri, S. Saxena, G. Iernetti, N. Dezhkunov, K. Yoshino, Measurement of the intensity of sonoluminescence, subharmonic generation and sound emission using pulsed ultrasonic technique, Jpn. J. Appl. Phys. Part 1 (Regular Papers Short Notes and Review Papers) 41 (8) (2002) 5329–5331.
[18] E.A. Neppiras, Subharmonic and other low-frequency emission from bubbles in sound-irradiated liquids, J. Acoust. Soc. Am. 46 (3) (1968) 587–601.
[19] W.G. Pitt, Defining the role of ultrasound in drug delivery, Am. J. Drug Deliv. 1 (1) (2003) 27–42.
[20] W.G. Pitt, G.A. Husseini, B.J. Staples, Ultrasonic drug delivery — a general review, Expert Opin. Drug Delivery 1 (1) (2004) 37–56.
[21] A. Marin, H. Sun, G.A. Husseini, W.G. Pitt, D.A. Christensen, N.Y. Rapoport, Drug delivery in pluronic micelles: effect of high-frequency ultrasound on drug release from micelles and intracellular uptake, J. Control. Release 84 (1) (2002) 39–47.
[22] S. Daniels, D. Blondel, L.A. Crum, G.R. ter Haar, M. Dyson, Ultrasonically induced gas bubble production in agar based gels: Part I. Experimental investigation, Ultrasound Med. Biol. 13 (9) (1987) 527–539.
[23] R.J. Urick, Principles of Underwater Sound, 3 ed., McGrawHill Book Company, San Francisco, 1983, p. 423.
[24] M.W.A. Kuijpers, D. van Eck, M.F. Kemmere, J.T.F. Keurentjes, Cavitation-induced reactions in high-pressure carbon dioxide, Science 298 (5600) (2002) 1969–1971.
[25] H.G. Flynn, C.C. Church, Transient pulsations of small gas bubbles in water, J. Acoust. Soc. Am. 84 (5) (1988) 1863–1876.
[26] R.E. Apfel, C.K. Holland, Gauging the likelihood of cavitation from short-pulse, low-duty cycle diagnostic ultrasound, Ultrasound Med. Biol. 17 (2) (1991) 179–185.
[27] S. Barnett, Thresholds for nonthermal bioeffects: theoretical and experimental basis for a threshold index, Ultrasound Med. Biol. 24 (S1) (1998) S41–S49.
[28] P.E. Huber, M.J. Mann, L.G. Melo, A. Ehsan, D. Kong, L. Zhang, M. Rezvani, P. Peschke, F. Jolesz, V.J. Dzau, K. Hynynen, Focused ultrasound (HIFU) induces localized enhancement of reporter gene expression in rabbit carotid artery, Gene Ther. 10 (18) (2003) 1600–1607.
[29] K. Hynynen, N. McDannold, H. Martin, F.A. Jolesz, N. Vykhodtseva, The threshold for brain damage in rabbits induced by bursts of ultrasound in the presence of an ultrasound contrast agent (Optison (R)), Ultrasound Med. Biol. 29 (3) (2003) 473–481.
[30] D.L. Miller, R.M. Thomas, Thresholds for hemorrhages in mouse skin and intestine induced by lithotripter shock waves, Ultrasound Med. Biol. 21 (2) (1995) 249–257.
[31] S. Mitragotri, J. Farrell, H. Tang, T. Terahara, J. Kost, R. Langer, Determination of threshold energy dose for ultrasound-induced transdermal drug transport, J. Control. Release 63 (1–2) (2000) 41–52.
[32] S. Mitragotri, D. Ray, J. Farrell, H. Tang, B. Yu, J. Kost, D. Blankschtein, R. Langer, Synergistic effect of low-frequency ultrasound and sodium lauryl sulfate on transdermal transport, J. Pharm. Sci. 89 (7) (2000) 892–900.
[33] A.H. Saad, A.R. Williams, Effects of therapeutic ultrasound on clearance rate of blood borne colloidal particles in vivo, Br. J. Cancer 45 (Suppl. V) (1982) 202–205.
[34] K. Tachibana, S. Tachibana, Albumin microbubble echo-contrast material as an enhancer for ultrasound accelerated thrombolysis, Circulation 92 (5) (1995) 1148–1150.
[35] A. Tezel, A. Sens, J. Tuchscherer, S. Mitragotri, Frequency dependence of sonophoresis, Pharm. Res. 18 (12) (2001) 1694–1700.
[36] W.L. Nyborg, Biological effects of ultrasound: development of safety guidelines: Part II. General review, Ultrasound Med. Biol. 27 (3) (2001) 301–333.
[37] A. Tezel, A. Sens, S. Mitragotri, Investigations of the role of cavitation in low-frequency sonophoresis using acoustic spectroscopy, J. Pharm. Sci. 91 (2) (2002) 444–453.
[38] S. Mitragotri, Synergistic effect of enhancers for transdermal drug delivery, Pharm. Res. 17 (11) (2000) 1354–1357.
[39] J.A. Rooney, Hemolysis near an ultrasonically pulsating gas bubble, Science 169 (1970) 869–871.
[40] N. Rapoport, D.A. Christensen, H.D. Fain, L. Barrows, Z. Gao, Ultrasound-triggered drug targeting of tumors in vitro and in vivo, Ultrasonics 42 (2004) 943–950.
[41] E.A. Neppiras, Acoustic cavitation, Phys. Rep. 61 (1980) 159–251.
[42] J.S. Allen, D.E. Kruse, P.A. Dayton, K.W. Ferrara, Effect of coupled oscillations on microbubble behavior, J. Acoust. Soc. Am. 114 (3) (2003) 1678–1690.
[43] W.L. Nyborg, Ultrasonic microstreaming and related phenomena, Br. J. Cancer 45 (Suppl. V) (1982) 156–160.
[44] D.J. Flannigan, K.S. Suslick, Plasma formation and temperature measurement during single-bubble cavitation, Nature 434 (7029) (2005) 52–55.
[45] N. Rapoport, A.I. Smirnov, A. Timoshin, A.M. Pratt, W.G. Pitt, Factors affecting the permeability of P. aeruginosa cell walls toward lipophilic compounds: effects of ultrasound and cell age, Arch. Biochem. Biophys. 344 (1) (1997) 114–124.
[46] N. Rapoport, W.G. Pitt, A.I. Smirnov, A.I. Timoshin, Bioreduction of tempone and spin-labeled gentamicin by gramnegative bacteria: kinetics and effect of ultrasound, Arch. Biochem. Biophys. 362 (2) (1999) 233–241.
[47] W.T. Shi, F. Forsberg, A. Tornes, J. Ostensen, B.B. Goldberg, Destruction of contrast microbubbles and the association with inertial cavitation, Ultrasound Med. Biol. 26 (6) (2000) 1009–1019.
[48] H.R. Guzman, A.J. McNamara, D.X. Nguyen, M.R. Prausnitz, Bioeffects caused by changes in acoustic cavitation bubble density and cell concentration: a unified explanation based on cell-to-bubble ratio and blast radius, Ultrasound Med. Biol. 29 (8) (2003) 1211–1222.