151d Ultrasonic Drug Release from Micelles Correlates with Subharmonic Emission

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Introduction Our group is studying the mechanisms of acoustically activated micellar drug delivery. We previously reported that micelles of Pluronic P-105 were capable of sequestering doxorubicin (Dox), thereby protecting HL-60 cells from damage, and that upon exposure to 70-kHz ultrasound the viability of these HL-60 cells was significantly reduced (1). Our hypothesis is that ultrasound releases drug from the micelles, which has been supported by further experiments with 20 and 70 kHz ultrasound (2). These experiments showed that amount released increased with increasing power density and decreased with frequency, suggesting that cavitation is involved. A common indicator of cavitation is the appearance of a subharmonic signal in the acoustic spectrum generated by the vibrations of the bubbles in the system. Here we present the relationship between the subharmonic emission and drug release from non-stablized and stabilized micelles.

Methods A custom ultrasonic exposure chamber with fluorescence detection was used to measure the release of Dox from Pluronic and two different formulations of stabilized micelles. An argon-ion laser was directed into an acoustically transparent tube containing the solution to be sonicated. Dox molecules were excited at 488 nm, and their fluorescence was then collected using a coaxial fiber optic collector. The detector signal was digitized for computer storage and processing. Dox fluoresces well in a hydrophobic environment (i.e., the micelle interior); but its fluorescence is partially quenched by water when it is released from the micelle. By calibrating the fluorescence of Dox in PBS and in 10% Pluronic solution, one can calculate the amount of Dox ultrasonically released into water from the decrease in fluorescence. Ultrasound at 70 kHz was generated in a Sonicor 100 bath (Copiague, NY). The intensity was controlled using a variac and monitored using a calibrated hydrophone (Bruel and Kjaer 8103, Decatur, GA). This hydrophone signal was then directed to a spectrum analyzer (Agilent E4401B) from which the acoustic spectrum was obtained.

Results and Discussion Figure 1 shows the acoustic intensity of the subharmonic signal correlated with drug release from non-stabilized micelles (the stabilized cases displayed similar behavior), calculated from change in fluorescence. In general, as the subharmonic intensity increases, so does the drug release. A threshold of release is evident for all types of carriers, which corresponds to the appearance of a visible subharmonic peak above the noise in the acoustic spectrum.

Both the onset of release and the appearance of the subharmonic exhibit a threshold of about 0.28 W/cm^2 of power density delivered. This behavior is analogous to the thresholds for both cavitation and biological effects of ultrasound reported in the literature. Whether the subharmonic signal is caused by stable or collapse cavitation remains controversial, however, and investigators have proposed equally plausible explanations for both cases. Since in this system it is associated with drug release, an understanding of the acoustic phenomena (type of cavitation) responsible for the subharmonic signal helps elucidate the mechanism of release from the carriers.

Stable cavitation creates shear forces near the bubble surface that may be strong enough to break open a loosely aggregated micelle of surfactant and expose its contents to the aqueous environment. Nevertheless, there is no direct evidence, theoretical or experimental, relating such strong shear forces to the observation of a subharmonic bubble vibration. Leighton (3) explains that a bubble can produce a subharmonic signal due to a prolonged expansion phase immediately preceding a delayed collapse phase of the cavitation event. Moreover, the bubble emits a shock wave upon implosion, which would then create high shear stresses as it expands through the liquid. In this case, drug release is induced from micelles not immediately adjacent to the collapsing bubble. Additionally, the shock wave produced

contains a wide spectrum of frequencies, reflected in 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. We suggest that this increase in background acoustic emission establishes the connection between collapse cavitation, the appearance of the subharmonic, and drug release from the micelles.

References 1. Husseini, G. A., El-Fayoumi, R. I., O'Neill, K. L. *et al.* (2000) *Cancer Letters* **154, 211-216.** 2. Husseini, G.A., Myrup, G.D., Pitt, W. G. *et al.* (2000) *Journal of Controlled Release* **69, 43-52.** 3. Leighton, T.G. (1994) *The Acoustic Bubble,* London: Academic Press. **613.**

Figure 1. Percent Dox release from Pluronic micelles correlated with subharmonic peak height.