I. INTRODUCTION

Over a range of conditions, ultrasound can transport molecules into viable cells by a mechanism believed to involve the transient disruption of cell membranes. Ultrasound has been demonstrated to deliver fluorescent dextran molecules (Fechheimer et al., 1986; Miller et al., 1999), genetic material (Bao et al., 1997; Greenleaf et al., 1998), and chemotherapeutic compounds (Saad and Hahn, 1987; Harrison et al., 1996) into viable cells. Involving a related mechanism, molecular uptake has also been observed following exposure of cells to lithotripsy shock waves (Holmes et al., 1992; Prat et al., 1993). Although these and other studies highlight the potential to use ultrasound for drug delivery, the quantitative dependence of bioeffects on acoustic parameters are insufficiently understood. To gain a better understanding of ultrasound and its biological effects, this study measured the dependence of bioeffects on acoustic pressure, exposure time, and pulse length by quantifying molecular uptake and cell viability on a cell-by-cell basis in two cell lines. Our hypothesis is that bioeffects correlate with acoustic energy exposure (J/cm²). Energy exposure may in turn relate to the strength of cavitation produced by ultrasound.

Ultrasound-mediated bioeffects are generally believed to be caused by cavitation, in the absence of ultrasonic heating (Carstensen et al., 1993). Cavitation is typically generated through activation of small dissolved gas nuclei by an acoustic pressure field. These nuclei, which grow through rectified diffusion, may oscillate and implode violently, thereby releasing a burst of energy that may be sufficient to disrupt cell membranes. Under some conditions, cavitation can cause irreversible cell damage resulting in cell death (Miller et al., 1996). Under other conditions, however, cavitation may reversibly disrupt cell membranes and thereby permit the entry of molecules into cells. It is the later phenomenon that we seek to achieve and control for drug delivery and other applications.

Other mechanical methods, such as cell scraping (McNeil, 1989) and syringe loading (Clarke and McNeil, 1992), are believed to apply shear forces that transiently disrupt cell membranes, which permit large molecules and genes to enter into cells. Electrical methods, such as electroporation (Chang et al., 1992), have also been employed to transport molecules across reversibly-disrupted cell membranes. These techniques are believed to physically disrupt cell membranes by a process independent of drug chemistry and, thus, may be employed to deliver a wide variety of drugs or genes. However, these mechanical and electrical techniques are generally invasive if applied in vivo. In contrast, ultrasound can be focused noninvasively from outside the body through the use of focused transducers. Local introduction of contrast agents may improve targeting further by lowering the pressure threshold required for cavitation in specified regions of the body (Holland and Apfel, 1990; Miller and Thomas, 1995).

II. MATERIALS AND METHODS

A. Maintenance and preparation of cells

DU145 human prostate cancer cells (DU145; American Type Culture Collection, item no. HTB-81, lot no. 1145858) were cultured as monolayers in a humidified atmosphere of 95% air and 5% CO₂ at 37 °C in RPMI-1640 media, supplemented with 10% (v/v) heat inactivated fetal bovine serum and 100-μg/ml penicillin-streptomycin (Cellgro, Mediatech, Herndon, VA). Human aortic smooth muscle cells (AoSMC; Clonetics, catalog no. CC-2571, lot no. 7F0787) were initi-
ated from frozen stock and harvested at passage 7 prior to each experiment. They were cultured as monolayers in a humidified atmosphere of 95% air and 5% CO$_2$ at 37°C in MCDB-131 media supplemented with 10% (v/v) heat inactivated fetal bovine serum, 100-μg/ml penicillin-streptomycin, and 2-mM L-glutamine (Cellgro).

Both cell types were harvested prior to each experiment by trypsin/EDTA (Cellgro) digestion at 80%–90% monolayer confluence and centrifuged at 1000 g for 6 min. The supernatant was discarded and cell pellets were resuspended in cell media to a concentration of 1 × 10^6 cells/ml, as determined by a haemocytometer (model 3200, Hauser Scientific, Horsham, PA) or Coulter Counter (Coulter Multisor II, Beckman Coulter). Cell radii were also determined using a Coulter Counter. Cell volumes were then calculated assuming the cells were spherical while in suspension.

Calcein (623 Da, radius = 0.6 nm; Molecular Probes, Eugene, OR, catalog no. C-481), a green-fluorescent molecule that cannot cross intact cell membranes, was used to quantitatively monitor the transport of molecules across the membranes of viable cells. Calcein was added to the cell suspension to attain a final concentration of 10^6 bubbles/ml; approximately 1.1 × 10^7 bubbles/ml; bubble diameter = 2.0–4.5 μm).

**B. Experimental equipment**

Ultrasound was generated by a submersible focused piezoceramic transducer (Techno Scientific, Woodbridge, Ontario, Canada) with a 3-in. diameter. The transducer had a 3½-in. focal length and a (~6-dB intensity area) beam width of 3 mm at the focal beam point. A 500-kHz sinusoid generated by a programmable waveform generator (Stanford Research Instruments, Sunnyvale, CA, model no. DS345) and amplified by a custom tone burst amplifier (Techno Scientific) powered and controlled the response of the transducer. As displayed in Fig. 1, the transducer was housed in a polycarbonate tank (12 × 11½ × 14½ in.) containing approximately 26 l of de-ionized, distilled, and partially degassed water at room temperature, 23–24°C. Mounted opposite the transducer, a 2-in.-thick acoustic absorber (SC-501 Acoustic Rubber, Sonic Concepts, WA) minimized standing wave formation. A degassing unit (Kaiser et al., 1996) and a 0.2-μm filtration unit (Fin-L-Filter, Cole-Parmer, Vernon Hills, IL) were operated for 3 h prior to each experiment to remove potential cavitation nucleation sites within the water bath. For calibration of the apparatus, spatial-peak–temporal-peak (SPTP) negative pressure ($P_{SPTP}^+$) was measured at the focal beam point using a 0.2-mm aperture PVDF membrane hydrophone (NTR Systems, Seattle, WA, model no. MHA200A) in the absence of a sample container.

The spatial-peak (SP) acoustic energy exposure ($E_{SP}$) measured in this study was approximated by the product of the spatial-peak-pulse-average (SPPA) acoustic intensity ($I_{SPPA}$) of a plane traveling wave and the total ultrasound exposure time, $t$.

$$I_{SPPA} = \frac{P^2}{\rho c},$$

$$E_{SP} = I_{SPPA} t,$$

where $P$ is rms pressure, $\rho$ the density of water, and $c$ the speed of sound in water. The following assumptions were made: (1) Ultrasound impinging on the cell sample was composed only of plane traveling waves, (2) the cell sample container was acoustically transparent, and (3) all of the acoustic energy measured at the focal beam point was transferred to the cell sample for all of the ultrasound conditions tested. In reality, however, a large fraction of the measured acoustic energy passes through the sample and is collected in the absorber. Thus, the energy actually delivered to cells is interpreted as being less than, but approximately proportional to, the reported energy exposure values.

**C. Ultrasound protocol**

Prior to ultrasound exposure, cell samples were slowly aliquoted via a 3-ml syringe (Becton Dickinson, Franklin Lakes, NJ) with a 22-gauge needle (Perkin Elmer, Foster City, CA) into 1.2-ml polyethylene transfer pipets (8.8 mm i.d., 0.3 mm wall thickness, and 3 cm height; Samco, San Fernando, CA, catalog no. 241). A metal rod attached to a three-way micropositioner (1-mm resolution, Velmix, Bloomfield, NY) was inserted into the pipet orifice to plug it closed and to hold and position the chamber in the focal beam point of the transducer (Fig. 1).

Ultrasound was delivered using pulses at a 6% duty cycle and with pulse lengths that varied from 20 μs to 60 ms. Exposure time was varied from 120 to 2000 ms. Since a 6% duty cycle was used, the actual duration of each experiment ranged from 2 to 34 s. $P_{SPTP}^+$ was varied between 0.6 and 3.0 MPa. “Sham” control exposures were conducted using the same protocol, but no ultrasound was applied. Postexposure cell samples were immediately transferred to 1.5-ml microcentrifuge tubes and left to incubate for 5 min at room temperature.
temperature to permit the cells to “recover” (Keyhani et al., 1998). The samples were then placed on ice until all of the samples were exposed (10–30 min).

Cell samples were washed and centrifuged (800 × g, 4 min, Eppendorf 5415C, Brinkman, Westbury, NY) three times to remove calcein present in the extracellular fluid (i.e., supernatant). Cell pellets were resuspended to a final volume of 0.5 ml in Dulbecco’s phosphate buffered saline (Cellgro) and incubated for at least 10 min with red-fluorescent propidium iodide (PI) solution (Molecular Probes, catalog no. P-1304) to stain nonviable cells. Fluorescent calibration beads (Molecular Probes, catalog no. L-14821) were added at a concentration of 2.4 × 10^4 beads/ml to facilitate cell viability analysis, as described previously (Prausnitz et al., 1993).

To verify that ultrasound exposure did not produce thermal effects, the temperature rise in a sample was measured at the largest peak negative pressure and longest exposure duration used in this study (i.e., 3.0 MPa and 2000 ms). Using a digital thermometer (HI 98501, Hanna Instruments, Woonsocket, RI) placed in the sample immediately after ultrasound exposure, the temperature rise in the sample was found to be less than 1 °C. To determine if a small temperature rise might induce bioeffects, samples were quickly heated (without ultrasound exposure) in a 37 °C water bath to a temperature increase of 5 °C (i.e., from 24 to 29 °C). Analysis of the samples showed no difference between the heated samples and unheated controls (Student’s t-test p > 0.5), and thus any bioeffects observed in this study were considered to be nonthermal.

D. Quantification of bioeffects

Optical properties of the cell suspensions were measured using a FacsVantage SE flow cytometer with Cell Quest software (Becton Dickinson). A minimum of 20,000 viable cells, collected at a rate of 1700 cells/s, was analyzed per sample to ensure that a statistically significant population was collected. Cell samples were excited with a 488-nm laser (Enterprise II, Innova, Coherent, Palo Alto, CA). Light scatter, collected by two photodiodes (forward scatter and side scatter), was used to determine the size and shape of particles (e.g., cells, debris, microspheres) in the sample. Fluorescence measurements, collected by two photomultiplier tubes, were used to distinguish viable from nonviable cells (PI fluorescence, 665- to 685-nm bandpass filter) and to measure calcein uptake (calcein fluorescence, 515- to 545-nm bandpass filter).

Figure 2 is a typical forward scatter—PI fluorescence plot of the collected raw data. The plot distinguishes viable cells (a), dead cells (b), and fluorescent beads (c). Viable cells show weak fluorescence in the red channel due to autofluorescence, optical and electrical noise, and possible low-level staining by propidium iodide. By comparing the ratio of cells to fluorescent beads in each sample to that of the control samples, it can be determined if cells were destroyed or otherwise “lost” during the protocol (Prausnitz et al., 1993). Quantitative calibration beads (Flow Cytometry Standards Corporation, Fishers, IN, catalog No. 825) were used to convert calcein fluorescence into an average number of molecules delivered per cell (Prausnitz et al., 1993). The raw data collected with the flow cytometer were analyzed using Windows Multiple Document Interface (WINMDI; TSRI Flow Cytometry, San Diego, CA) flow cytometry software (Canetella et al., 2001).

E. Statistical analysis

At each condition tested, a minimum of three replicate data points was collected. Replicates were utilized to calculate experimental means and standard errors. One-way analysis of variance (ANOVA, α = 0.05) was performed when comparing three or more experimental conditions to a single factor. When two factors were compared, a two-way analysis of variance was used. A value of p < 0.05 was considered statistically significant.

The dependence of experimental data on acoustic energy exposure was described mathematically by fitting to a sigmoidal curve. The fit was optimized using the Microsoft EXCEL solver function (Microsoft, Redmond, WA). The sigmoidal dependence was selected as the appropriate functionality based on the observation that the extent of ultrasound’s effects on cells increased monotonically with acoustic energy above a low-energy threshold and below a high-energy plateau or saturation. A coefficient of determination (R^2) was used to quantify “goodness” of fit.

To identify trends in experimental data where no functionality was known or assumed, regression models based on restricted cubic splines (S-Plus, MATHSOFT, Seattle, WA) were used. “Goodness” of fit for each trend was determined using the multiple R^2 statistic, which represents the amount of variability in the response variable (e.g., uptake) that is explained by the fitted variable (e.g., pressure). A multiple R^2 of 1 indicates a perfect relationship between the fit and response variables, while a multiple R^2 of 0 indicates no relationship.
III. EXPERIMENTAL RESULTS

This study characterized the effects of acoustic pressure, total exposure time, and pulse length on human prostate cancer (DU145) and human aortic smooth muscle (AoSMC) cell suspensions. Quantitative analysis of the bioeffects (i.e., molecular uptake and cell viability) was performed using flow cytometry.

A. Acoustic pressure dependence

The effects of peak negative pressure over the range of 0.6–3.0 MPa on DU145 and AoSMC cells were studied using ultrasound exposures of different durations. As shown in Figs. 3(a) and (b), the number of calcein molecules delivered per viable cell generally increased with increasing pressure (Table I). Over the same range of acoustic conditions, cell viability generally decreased with increasing pressure [Figs. 3(c) and (d), Table I]. Together, these results show that large numbers of molecules (e.g., millions of molecules per cell) can be delivered into cells, but there can also be significant loss of cell viability.

Closer inspection of the data shows that a large (>50%) decrease in viability occurred between 1.6 and 3.0 MPa at 1- and 2-s exposure times, which suggests that high negative pressures (>1.6 MPa) and long exposure times (>1 s) may be less desirable for transiently disrupting viable cells. However, these conditions may be useful for applications where

<table>
<thead>
<tr>
<th>Exposure time (ms)</th>
<th>DU145 Uptake</th>
<th>DU145 Viability</th>
<th>AoSMC Uptake</th>
<th>AoSMC Viability</th>
</tr>
</thead>
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<td>120</td>
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<td>0.56</td>
<td>0.53</td>
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<tr>
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<td>0.93</td>
</tr>
<tr>
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<td>0.36</td>
<td>0.12</td>
<td>0.53</td>
<td>0.85</td>
</tr>
<tr>
<td>Average</td>
<td>0.47</td>
<td>0.67</td>
<td>0.48</td>
<td>0.71</td>
</tr>
</tbody>
</table>

TABLE I. Multiple $R^2$ values for restricted cubic spline fits (data from Figs. 3 and 4).

$^a$The dependence of uptake and viability on pressure is determined at five different exposure times (data from Fig. 3).

$^b$The dependence of uptake and viability on exposure time is determined at seven different pressures (data from Fig. 4).
high molecular uptake accompanied by significant cell death is beneficial, such as in targeted cancer chemotherapy.

B. Exposure time dependence

To better show the effect of ultrasound exposure time on cells, the data presented in Fig. 3 are replotted as shown in Fig. 4. In this set of experiments, exposure time was varied between 120 and 2000 ms. Figures 4(a) and (b) show that calcein uptake generally increased with exposure time (Table I). Similarly, cell viability generally decreased with exposure time [Figs. 4(c) and (d), Table I].

C. Pulse length dependence

Because pulse length was held constant at 60 ms in the experiments described previously, we conducted additional experiments in which the pulse length was varied between 0.02 ms (10 cycles/pulse) and 60 ms (30,000 cycles/pulse). As shown in Fig. 5, pulse length did not have a significant effect on molecule uptake or cell viability at the conditions studied (one-way ANOVA: $p=0.97$ for uptake and $p=0.42$ for viability).

D. Acoustic energy correlation

The families of curves shown in Figs. 3–5 indicate that bioeffects exhibit a strong dependence on both exposure time and acoustic pressure, but no dependence on pulse length. This suggests that energy exposure, which scales with exposure time and the square of pressure, but is independent of pulse length [see Eq. (2)], might also correlate with bioeffects and possibly serve as a single unifying parameter that could collapse all of the data into a single curve.

To test this hypothesis, we replotted all of the uptake and viability measurements shown in Figs. 3–5 versus applied acoustic energy exposure. As shown in Fig. 6, calcein uptake and cell viability correlated with energy, where each set of data collapsed into a single curve (restricted cubic spline multiple $R^2=0.76$ and 0.78 for viability of DU145 and AoSMC cells, respectively; multiple $R^2=0.61$ and 0.39 for uptake by DU145 and AoSMC cells, respectively). The observation that bioeffects generated over a broad range of ultrasound conditions all correlate with acoustic energy gives insight into possible mechanisms and can guide development of applications, as discussed in the following sections.

The above-stated statistical analysis could be enhanced by assigning a functionality to the dependence of bioeffects on energy exposure. We noted that the shape of each curve in Fig. 6 appears to be sigmoidal: At low energy both uptake and loss of viability show weaker dependence on energy; at moderate energies the bioeffects increase as strong functions of energy; and at high energy the bioeffects level off. For this reason, we used a sigmoidal function to mathematically describe the data in Fig. 6, which yielded the following:

$$N = N_{\text{max}}(1 - e^{-aE^{2/3}}),$$

$$V = 100\% (e^{-aE^{2/3}}),$$

where $N$ is the number of calcein molecules delivered per cell, $V$ is cell viability, $E$ is acoustic energy exposure [J/cm$^2$, as defined in Eq. (2)], $N_{\text{max}}$ is an empirically determined parameter.
upper limit for the number of molecules that can be delivered into each cell type, and the adjustable parameter \( \alpha \) empirically accounts for differences in cell type (\( N_{\text{max}} \) equals 1.2 \( \times 10^7 \) and 4.6\( \times 10^6 \), and \( \alpha \) equals \(-0.036\) and \(-0.057\) for DU145 and AoSMC cells, respectively).

When compared to the fits generated previously using the multiple functionalities permitted by restricted cubic splines, the goodness of fit using the sigmoidal relationships provided equally robust correlations for uptake [Eq. (3), \( R^2 = 0.59 \) and 0.34 for DU145 and AoSMC, respectively] and for viability [Eq. (4), \( R^2 = 0.76 \) and 0.79 for DU145 and AoSMC, respectively], indicating that sigmoidal fits are appropriate. Interestingly, the correlations shown in Figs. 4 and 5 have the same exponent for the energy dependence for both cell types (\( E^{2/3} \)), suggesting similarity in mechanism. A two-way ANOVA shows significantly different levels of uptake between DU145 and AoSMC as a function of increasing energy exposure (\( p < 0.01 \)). However, differences in viability were not significant between cell types as a function of increasing energy exposure (\( p = 0.98 \)). Future studies are needed to establish if the correlation with energy observed here also exists at other frequencies, contrast agent concentrations, experimental conditions, and in other cell types.

**E. Optimal exposure conditions**

For practical applications such as gene transfection, optimal ultrasound conditions may be those that maximize the product of the number of molecules delivered per viable cell (\( N \)) and cell viability (\( V \)). Conditions that yield the greatest \( NV \) would produce a cell population with the most molecules in the most viable cells. Previous work has suggested that \( NV \) correlates with degree of gene transfection for many cell types (P. Canatella, personal communication).

As shown in Fig. 7, \( NV \) generally increased with energy, reached a maximum, and then decreased with further increasing energy (Multiple \( R^2 = 0.17 \) and 0.43 for DU145 and AoSMC, respectively). This suggests that \( NV \) may be optimized within a defined energy window. At low energy levels, little uptake occurred and, at high energy levels, significant cell death occurred, each resulting in low \( NV \) values. The region of interest lies in between (i.e., combinations of low pressure-long exposures, high pressure-short exposures, and moderate pressure-medium duration exposures). Figure 7 shows that optimal \( NV \) was at approximately the same energy exposure for both cell lines (i.e., \( \sim 50 \) J/cm\(^2\)).

**IV. DISCUSSION**

**A. Correlation with energy exposure**

In this study, simultaneous measurements of molecular uptake and cell viability were performed on DU145 and AoSMC cells over a wide range of acoustic parameters. In support of our proposed hypothesis, bioeffects generally correlated with acoustic energy exposure regardless of the acoustic pressure, exposure time, and pulse length used. A similar finding was made by Mitragotri et al. (2000), who observed that acoustic energy exposure correlated with increased skin conductivity induced by exposure to 20-kHz ultrasound.

The dependence of bioeffects on energy exposure may be of use to suggest or validate mechanistic understanding, but is also relevant to practical applications. A dependence on energy gives an experimentalist or clinician considerable freedom to use different ultrasound conditions that produce the same energy. For example, one could use long, low-pressure exposures which require a less expensive transducer and power supply or similarly use short, high-pressure pulses of the same energy if extremely rapid (i.e., subsecond) effects are desirable. In addition, the correlation of acoustic energy with \( NV \), which is believed to be a marker of transfection, can guide optimization of gene delivery protocols. Further study is needed to determine if these results can be extended to tissue and the in vivo environment.

**B. Maximum levels of uptake**

Over the full range of acoustic conditions studied, average uptake never exceeded 1.2\( \times 10^7 \) molecules per DU145 cell or 4.6\( \times 10^6 \) molecules per AoSMC cell. Based on average cell volumes of 2200 and 2400 \( \mu \)m\(^3\) for DU145 and AoSMC cells, respectively (determined using a Coulter Counter), this corresponds to intracellular concentrations of approximately 9.1 \( \mu \)M in DU145 and 3.2 \( \mu \)M in AoSMC.
cells. Since the extracellular concentration was 10 μM, this indicates that approximate thermodynamic equilibrium with the extracellular solution was achieved in DU145 cells, although associated with large loss in viability. This finding contrasts with results for molecular uptake by electroporation, where “subequilibrium” uptake was always observed (Prausnitz et al., 1993; Canatella et al., 2001).

C. Cavitation-based mechanism

The observed dependence of bioeffects on energy suggests the possibility of a thermal mechanism of action. However, our data in combination with insight from the literature indicate that cavitation, rather than heat, is responsible for the observed effects. In this study, all experiments were performed with contrast agent (CA) present in the cell sample. CA is known to nucleate cavitation and thereby lower the pressure required to generate and sustain bubble activity (Holland and Apfel, 1990; Miller and Thomas, 1995). CA is not expected to influence thermal effects. As shown in Fig. 8, cell samples lacking CA experienced minimal loss of cell viability (one-way ANOVA \( p = 0.97 \)) (and insignificant uptake of molecules; data not shown) as opposed to cells exposed to ultrasound in the presence of CA (one-way ANOVA \( p < 0.001 \)).

This observation verified our *a priori* assumption about cavitation-induced bioeffects and is in agreement with others who have shown that CA can significantly enhance bioeffects produced by ultrasound (Miller et al., 1997; Greenleaf et al., 1998; Poliachik et al., 1999). As described in Sec. II, the possible role of thermal effects was further investigated by rapidly increasing cell sample temperature by 5 °C. This rise in temperature produced no statistically significant bioeffect on cells. When exposed to ultrasound, the temperature rise in the cell samples was always less than 1 °C. This lack of significant bulk heating and the need for CA indicates that cavitation mediated the observed bioeffects.

D. Lack of dependence on pulse length

Figure 4 showed that bioeffects were independent of pulse length over the range of conditions tested (10–30 000 cycles/pulse), in agreement with previous work (Brayman and Miller, 1999). However, this observation differs from other studies in which the degree of red blood cell hemolysis was shown to depend on pulse length (Ciavarino et al., 1981; Kober and Ellwant, 1989; Liu et al., 1998). Recent work performed in our lab using DU145 cells also demonstrated a dependence of bioeffects of pulse length (Cochran and Prausnitz, 2001). A possible explanation for this discrepancy may involve the presence of CA microbubbles; those studies that observed a pulse length dependence did not have CA present during insonification, while those that observed no dependence had CA in their samples. In the absence of CA, it may take a minimum amount of time or number of acoustic cycles within a pulse to nucleate and grow bubbles, suggesting that a minimum pulse length is needed. In contrast, Flynn and Church (1988) noted that cavitation could occur after just one acoustic cycle if appropriately sized nuclei are supplied, such as those provided by CA. Thus, if cavitation

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FIG. 6. Calcein uptake increased in (A) DU145 and (B) AoSMC cells with increasing acoustic energy exposure. Cell viability decreased in (C) DU145 and (D) AoSMC cells with increasing acoustic energy exposure. Ultrasound exposure durations were: \( \bigcirc = 120 \), \( \triangle = 240 \), \( \square = 540 \), \( \bullet = 1000 \), and \( \Delta = 2000 \) ms (data are from Figs. 3 and 4). Pulse length data (i.e., from Fig. 5) are represented by \( \Diamond \). Sigmoidal data fits are given by the solid lines [Eqs. (3) and (4)]. Data expressed as mean±SEM.
FIG. 8. The presence of 1.7% contrast agent (●) during insonation caused viability to decrease with increasing pressure at 2-s total exposure time and 60-ms pulse length. No effect was observed under the same ultrasound conditions in the absence of contrast agent (○). Data expressed as mean±SEM.

FIG. 7. The N V product (N = number of molecules delivered per cell, V = cell viability) increased, reached a maximum, and then decreased with increasing energy exposure in both (a) DU145 and (b) AoSMC cells. Ultrasound exposure durations were: ○ = 120, △ = 240, □ = 540, ■ = 1000, and Δ = 2000 ms. Data fits (solid line) were obtained from the product of Eqs. (3) and (4). Data expressed as mean±SEM.

can be induced at the onset of ultrasound exposure through the use of CA, then pulse length appears not to be an important parameter.

V. CONCLUSIONS

By exposing DU145 and AoSMC cells to a broad range of ultrasound conditions, we determined that ultrasound can deliver millions of calcein molecules into viable cells at an intracellular concentration that approaches the extracellular concentration. In both cell types, uptake increased and viability decreased with increasing ultrasound pressure and exposure time; pulse length, however, did not influence bioeffects in the samples, which were preseeded with cavitation nuclei. All of the data collapsed into a single curve when plotted as a function of acoustic energy exposure, suggesting that this may be predictive of ultrasound’s nonthermal bioeffects. Based on a criterion of maximizing the product of molecular uptake and cell viability, optimal ultrasound conditions were identified on the basis of an optimal acoustic energy exposure. Because contrast agent was needed to observe bioeffects and heating was negligible, we propose that cavitation mediated by acoustic energy exposure is the mechanism of ultrasound’s effects on cells observed in this study.

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Canatella, P. J. (personal communication).


