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Effects of Stress Waves on Cells

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ABSTRACT

Laser induced stress waves are being used in a variety of medical applications, including drug delivery and targeted tissue disruption. Stress waves can also be an undesirable side effect in laser procedures such as ophthalmology and angioplasty. Thus, a study of the effects of stress waves on a cellular level is useful. Thermoelastic stress waves were produced using a Q-switched frequency-doubled Nd:YAG laser (λ=532nm) with a pulse duration of 4 ns. The laser radiation was delivered to an absorbing media. A thermoelastic stress wave was produced in the absorbing media and propagated into plated cells. The energy per pulse delivered to a sample and the spot size were varied. Stress waves were quantified. We assayed for cell viability and damage using two methods. The laser parameters within which cells maintain viability were investigated and thresholds for cell damage were defined. A comparison of cell damage thresholds for different cell lines was made.

Key Words: stress wave, cell damage, photoacoustic

INTRODUCTION

Short pulse lasers are used in a number of clinical procedures, including lithotripsy, ophthalmology and drug delivery. Pressure transients produced by these short pulse lasers may produce damage to surrounding cells and tissues. Stress waves are produced when optical energy is absorbed into an appropriate medium. If the deposition of this energy is less than the stress confinement time, a wave is produced.

There has been some research done in this field previously, most notably at Wellman Laboratories of Photomedicine. They have used excimer lasers, with rise times from 10-25 ns in their experiments on stress waves and cell death. They have reported stresses up to kbars, though they have mainly published results on the compressive components of the waves. Fluences ranged from 90-560 mJ/cm^2. Their results were cell-type dependent.[1]

MATERIALS AND METHODS

Figure 1 shows the experimental arrangement. A slide coverslip with black enamel paint on the underside was placed onto a second coverslip. Water-based gel placed between the paint and glass assured good acoustic coupling. This second coverslip had cells plated on the underside. Stress waves were produced by the deposition of optical energy into the black paint, which is in turn vaporized, propagating a stress wave. The approximate distance between the absorption of the laser and the target cells was 450 μm. This experimental arrangement prevented any measurable temperature rise and optical energy to reach the cells.

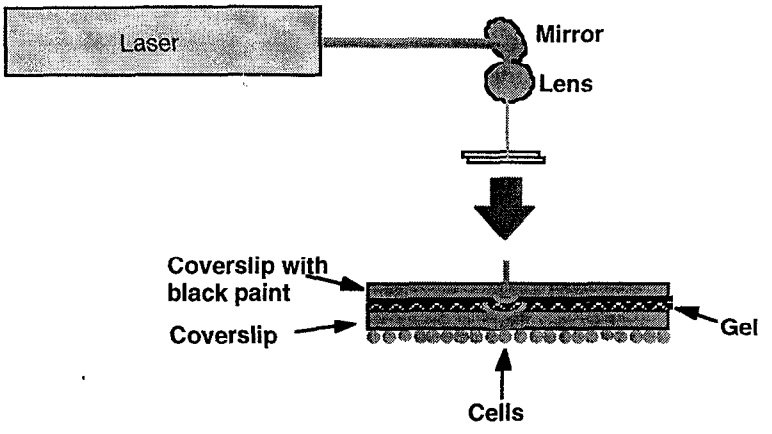

Figure 1. Experimental Set-up

The laser source was a Q-switched, frequency-doubled Nd:YAG laser (Spectra Physics, Mountain View, CA). The wavelength was 532 nm and the beam profile was Gaussian. Pulse duration was approximately 4 ns. Energy per pulse was varied from 2.13 mJ to 217 mJ, using neutral density filters. Spot size was also varied by focusing the beam and moving the target to discrete distances. Spot sizes obtained varied from 440 μm to 2400 μm. as measured by photo-sensitive paper. Consequently, the fluence range was 226 mJ/cm^2 to 6060 mJ/ cm^2.

We estimated the stress confinement time of our experimental arrangement to be 30 ns ($\tau_s=d/c_s$). This was calculated using a penetration depth, d, of 50 μm and 1500 m/s as the speed of sound, c_s, in our medium. Since the deposition of our energy occurred within 4 ns, the energy was stress-confined.

The pressure magnitude in the stress waves was measured using a PVDF (polyvinylidene fluoride) hydrophone (NTR Systems, Seattle WA). The hydrophone has a relatively flat frequency response from 0-20 MHz and the sensing area is 1 mm in diameter. Figure 2 shows a typical pressure profile obtained with the hydrophone. The hydrophone was placed at varying distances from the target and a plot of distance vs. pressure was obtained.

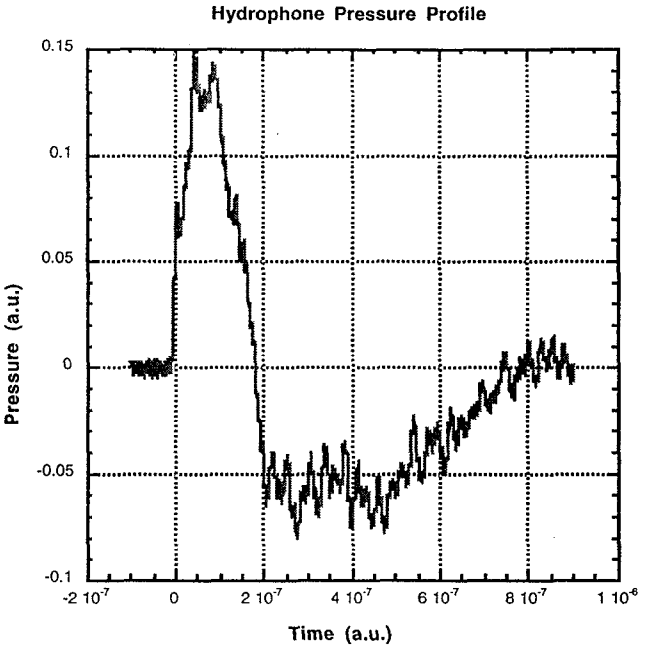

Figure 2. Pressure profile

Three different cell lines (ATCC, Rockville, MD) were used as targets: 769P (human renal adenocarcinoma), NCTC (mouse fibroblasts from connective tissue), and MES-SA (human uterine sarcoma). All cells were grown under standard cell culture techniques, in a 37°C, 5% CO_2 incubator. Cells were passed and plated onto slide coverslips that were coated with poly-L-lysine to promote adhesion. Cells were then placed back in the incubator, allowed to reach near-confluency and used as targets in the laser experiments.

After laser irradiation, cells were returned to the incubator for 12 hours and then were assayed for cell death using two methods: fluorescence microscopy and Trypan Blue. The fluorescence microscopy assay involved the use of the LIVE/DEAD Viability/Cytotoxicity kit (Molecular Probes, Eugene, OR), which is comprised of calcein-AM and ethidium homodimer-1, both of which fluoresce weakly in the media. Figure 3 shows the mechanism of the assay. The AM portion of the calcein-AM molecule facilitates transport across the plasma membrane. Once inside a live cell, the AM is cleaved by intracellular esterases and calcein becomes highly fluorescent (green). Ethidium homodimer-1 cannot enter cells through intact membranes. However, in a dead cell with a compromised membrane, ethidium homodimer-1 enters the cell, binds to the DNA and increases its red fluorescence intensity 40-fold. Calcein-AM also enters the dead cell but the AM is not cleaved and it remains virtually non-fluorescent. A solution of 150 µl of 4 µM calcein-AM and 4 µM ethidium homodimer-1 was added to the coverslips and allowed to incubate at room temperature for 30 minutes. Coverslips were mounted on slides and observed under a fluorescent microscope (Zeiss). Trypan Blue staining was performed in the usual manner. Trypan Blue enters cells with compromised membranes and is unable to enter those with intact membranes. Both methods produced similar results.

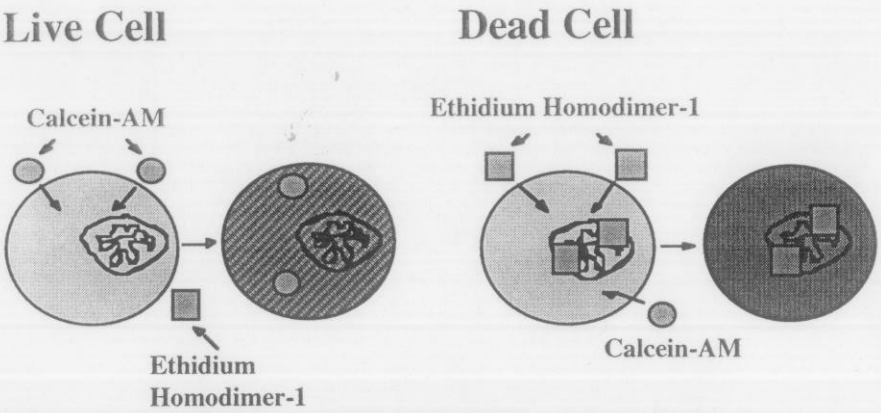

Figure 3. Fluorescence Microscopy Assay

RESULTS

The stress waves produced had both a positive (compression) and negative (tension) component. This results from reflections off of the glass-air interface. The magnitude of the tensile portion of the wave was approximately half the magnitude of the compression portion of the wave. Figure 4 shows the percent of samples demonstrating some degree of damage as a function of the fluence produced, for the NCTC target cells. The laser-treated samples are grouped according to average spot size. No damaged samples were observed at the smallest spot size (560 μm), regardless of the resulting fluences (1400, 3480, and 6060 mJ/cm^2). However, at the larger spot sizes, a positive correlation was observed between the fluence produced and the percent of samples that were damaged. For the largest average spot size (2000 μm), there was no damage observed at the lowest fluences (226 and 483 mJ/cm^2). Damaged samples were observed at the higher fluences (701, 913, and 1200 mJ/cm^2). For the medium average spot size (960 μm), no damage was observed at the lowest fluence (424 mJ/cm2), but damage was noted at 829, 1180, and 2590 mJ/cm^2. Figure 5 shows the correlation between fluences and the pressure differential at the distance from the target that the cells were placed. Damaged samples were observed at the two larger spot sizes between 200 bar and 400 bar. At approximately 400 bar, 100 percent of the samples were damaged. The 2000 μm spot size had resulting pressures up to 554 bar, though damage peaked at 400 bar. The 960 μm spot size had resulting pressures up to 432 bar.

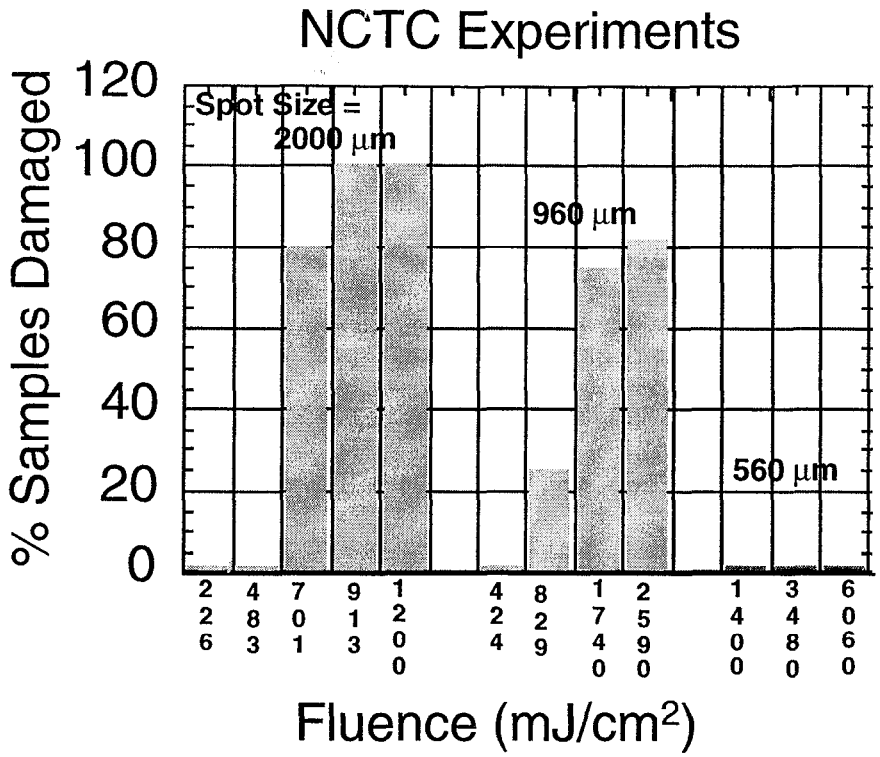

Figure 4. Fluence vs. % Samples Damaged

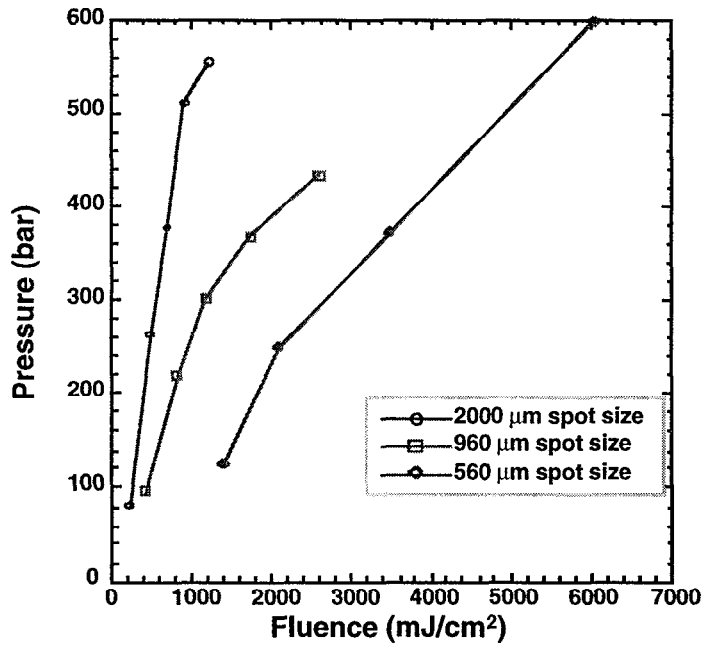

Figure 5. Fluence vs. Pressure at Cells

At times, we observed a hole in the cell layer, visible to the naked eye, immediately after laser treatment. In other instances, a hole was not observed immediately following treatment, but was observed during the cell death assays. A combination of two events may be happening. The cells may be knocked

off the coverslip by the pressure of the stress waves. Alternatively, cells may be dying during the 12 hour incubation and floating off of the coverslip, resulting in a hole during the assay. Dead cells typically lose their adhesion to their substrate. The three cell lines used as targets had similar thresholds for damage.

DISCUSSION

We believe that spot size effects were responsible for the differences in damage that we observed. With a small spot size, the stress wave produced propagates in a spherical manner almost from the outset. The peak pressure in a spherical wave falls off in a 1/r manner. However, a larger spot size will initially produce a planar wave, in which the peak pressure falls off more slowly, in a linear fashion. This planar wave eventually evolves into a spherical wave, due to defraction, and the pressure begins to fall off at a rate of 1/r. This would account for the higher number of samples damaged with the larger spot sizes, and lower fluences, and the lack of damage observed at the smaller spot sizes. To prevent damage to the hydrophone, the proximity to the target was limited to 1 mm. For the larger spot size, the pressure falls off at a rate close to 1/r, whereas at closer than 2 mm, the pressure seems to fall away at a linear rate. With the smaller spot size, the pressure falls off at a rate that closely approximates 1/r.

The existence of a tensile component to the stress wave accounts for the differences between our results and those obtained at Wellman. The cell damage threshold for tensile waves has been found to be significantly lower than that for solely compressive waves.[2] Our findings are relevant to fiber-delivered applications, in which the resulting stress waves have both a compressive and tensile component. Cavitation was probably also present in our experimental set-up. Water fails at approximately 10-100 bar, negative component.

We intend to further quantify the cell death and damage observed, using assays to detect earlier stages of cell death than membrane integrity. In addition, temporal effects on cell damage, obtained by varying the incubation time post-treatment, will be explored. Obtaining data on the damage caused by the different components of the wave is also of interest. Spot size effects, multiple pulses, and pulse duration differences will be investigated.

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