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The overall objective of this project was to study the role of mechanical effects in producing retinal injury as part of a larger Air Force program on ultrashort laser pulse ocular injury. Laser-induced stress transients were generated by the ablation of a polyimide target by an excimer laser in order to study damage due only to stress waves, excluding confounding factors such as cavitation. The model for ocular injury consisted of retinal pigment epithelium (RPE) cells in vitro. The response of RPE cells to compressive waves has been determined using a dye exclusion assay to determine cell killing. The susceptibility of RPE cells to damage by stress waves varies with cell line. Transformed retinal pigment epithelium cells are more susceptible than normal ones. Saturation of damage versus number of stress wave pulses is observed, and a threshold-like behavior of cell killing versus stress is found. A system for generating purely tensile stress waves was developed and initial results showing that tensile stress waves are far more damaging to RPE cells than compressive ones were obtained. In order to characterize the propagation of stress waves in ocular media we ahve used picosecond transient grating spectroscopy to determine the acoustic attenuation and the sound velocity of the vitreous and the lens of the

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bovine eye in the 925-1020 Mhz range. These experiments indicated that at this frequency the sound velocity of bovine vitreous can be well approximated by that of water, but the acoustic attenuation coefficient is much higher than would be extrapolated from published low-frequency data.

AIR FORCE OFFICE OF SCIENTIFIC RESEARCH

FINAL TECHNICAL REPORT

Title:	"Ultrashort Laser Pulse Effects in Ocular and Related Media: Laser-Induced Shock Wave Propagation and Retinal Damage"
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SUMMARY

The overall objective of this project was to study the role of mechanical effects in producing retinal injury as part of a larger Air Force program on ultrashort laser pulse ocular injury . Laser-induced stress transients were generated by the ablation of a polyimide target by an excimer laser in order to study damage due only to stress waves, excluding confounding factors such as cavitation. The model for ocular injury consisted of retinal pigment epithelium (RPE) cells in vitro. The response of RPE cells to *compressive* waves has been determined using a dye exclusion assay to determine cell killing. The susceptibility of RPE cells to damage by stress waves varies with cell line. Transformed retinal pigment epithelium cells are more susceptible than normal ones. Saturation of damage versus number of stress wave pulses is observed, and a threshold-like behavior of cell killing versus stress is found. A system for generating purely *tensile* stress waves was developed and initial results showing that tensile stress waves are far more damaging to RPE cells than compressive ones were obtained. In order to characterize the propagation of stress waves in ocular media we have used picosecond transient grating spectroscopy to determine the acoustic attenuation and the sound velocity of the vitreous and the lens of the bovine eye in the 925-1020 Mhz range. These experiments indicated that at this frequency the sound velocity of bovine vitreous can be well approximated by that of water, but the acoustic attenuation coefficient is much higher than would be extrapolated from published low-frequency data.

REPORT

The support provided by AFOSR F49620-93-1-0290 resulted in the publication of three papers, with a fourth submitted. Our research is summarized below.

Damage to in vitro retinal pigment epithelium (RPE) cells by compressive pressure waves (stress transients) was studied. Normal and transformed human retinal pigment epithelium cell lines were used. The cells were imbedded in a gel to prevent motion and cavitation and located in a thin layer at the bottom of a pipette tube closed at one end by a polyimide film. Stress transients were generated by pulsed excimer laser (193 nm and 248 nm wavelength) ablation of the polyimide film. Cell survival, compared to that of unirradiated cells, was assessed by counting surviving cells. The stress was varied from 300 to 740 bars and the number of shock wave pulses applied varied from 5 to 150.

Cell survival decreased sharply at the higher stresses but some cells always survived. The lowest survival rate was 50 %. Increasing the number of shock wave pulses did not increase cell killing after 20 pulses, demonstrating a saturation effect. In contrast to the transformed cell line, normal cells could not be killed at the highest stress available to us.

Conclusions: The susceptibility of RPE cells to damage by stress waves varies with cell line. Transformed retinal pigment epithelium cells are more susceptible than normal ones. Saturation of the damage versus number of pulses is observed and a threshold-like behavior for cell killing versus stress is found. Because at least 50 % of the cells survived, normal cell growth can serve to replenish damaged cells.

The propagation of stress waves in ocular media was studied using picosecond transient grating spectroscopy. Measurements of the acoustic attenuation and the sound velocity were made on a number of liquids, transparent ocular materials (vitreous and lens of the bovine eye) and biological fluids (whole blood) at frequencies between 925 and 1020 MHz using a picosecond thermal grating. Sound velocity and attenuation measurements of liquids (e.g. methanol, ethanol) agree very well with those reported in the literature, validating the method. The sound velocity in the biological materials studied also agrees with the reported values in the literature. On the other hand, the attenuation coefficients measured for biological materials, 2000-5000 dB/cm, are much higher than would be extrapolated from published low frequency data. These measurements provide a basis for future Air Force mathematical modeling of acoustic propagation and damage in the eye.

A system for generating purely tensile stress waves was developed in order to compare the effects of compressive and tensile stresses on RPE cells. Preliminary results showing that tensile stress waves are far more damaging to RPE cells than compressive ones have been obtained.

Tensile stress waves are produced by using a plastic prism to internally reflect a compressive stress wave generated by laser ablation of a target on the surface of the prism. Plastic (polyethylene) is used because it provides an excellent acoustic impedance match to water, which is the primary component of cells. In water pure tensile stresses of up to -200 bars with a rise time of 20 ns and a duration of 100 ns are achieved. Previously, methods of generating tensile stresses have generally produced tensile waves accompanied by compressive ones. This marks the first time that pure tensile stresses with very short rise times have been produced.

Experiments on the effect of tensile waves on RPE cells were not completed during the contract period because of substantial problems with laser sources. These have now been resolved and cell experiments will be conducted using a new excimer high-energy laser and polyimide targets. Completion of these experiments under other funding is expected in early 1997.

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