Wavelength Dependence of Cell Cloning Efficiency after Optical Trapping

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ABSTRACT A study on clonal growth in Chinese hamster ovary (CHO) cells was conducted after exposure to optical trapping wavelengths using Nd:YAG (1064 nm) and tunable titanium-sapphire (700–990 nm) laser microbeam optical traps. The nuclei of cells were exposed to optical trapping forces at various wavelengths, power densities, and durations of exposure. Clonal growth generally decreased as the power density and the duration of laser exposure increased. A wavelength dependence of clonal growth was observed, with maximum clonability at 950–990 nm and least clonability at 740–760 nm and 900 nm. Moreover, the most commonly used trapping wavelength, 1064 nm from the Nd:YAG laser, strongly reduced clonability, depending upon the power density and exposure time. The present study demonstrates that a variety of optical parameters must be considered when applying optical traps to the study of biological problems, especially when survival and viability are important factors. The ability of the optical trap to alter either the structure or biochemistry of the process being probed with the trapping beam must be seriously considered when interpreting experimental results.

INTRODUCTION

Ashkin (1970) first described the optical trapping of micrometer-sized dielectric particles by using two opposing laser beams. Ashkin and his co-workers were also the first to propose and demonstrate the use of optical traps for biological applications (Ashkin, 1980; Ashkin et al., 1987; Ashkin and Dziedzic, 1989). Since then, laser-based optical traps have been used to study sperm motility (Tadir et al., 1989, 1990); to measure the compliance of the bacterial flagellum (Block et al., 1990); to study cellular motors (Kuo and Sheetz, 1993; Block et al., 1990); for cell sorting (Buican et al., 1987, 1989); to manipulate objects inside plant cells (Ashkin and Dziedjic, 1989); to study DNA relaxation after the attachment of microbeads to the DNA molecule (Perkins et al., 1994); to bring two cells together for laser-induced cell fusion (Wiegand Steubing et al., 1991); and to manipulate and study chromosome movement in mitotic cells (Berns et al. 1989, 1992; Liang et al., 1991, 1993, 1994). The diversity of recent biological studies employing optical trapping suggests a wide application of this novel tool for the manipulation of living cells and cellular organelles. In addition, there are now commercially available systems for optical trapping, thus making this technology generally available to the biological research community.

However, as is often the case with a new technology, its application often precedes a full and comprehensive understanding of the technology itself. As a result, the technology may have significant side effects on the objects/systems that it is being used to study. For example, it has been shown recently that optical traps can produce a temperature increase at the trap focal point as a function of power density and duration of exposure (Liu et al., 1995). Exposure of CHO cells to a 1064-nm optical trap with an incident power of 100 mW, corresponding to a power density of $\sim 3 \times 10^7$ W/cm^2, resulted in a temperature rise of 1.15°C/100 mW laser power. Additionally, in an earlier study on mitotic chromosomes (Vorobjev et al., 1993) we observed a wavelength dependency in the formation of abnormal chromosome bridges, with maximum bridging occurring at trapping wavelengths of 760–765 nm. This was the first study demonstrating wavelength-specific adverse effects of optical trapping. In light of this fact and the growing use of optical traps in many different areas of cellular research, we have undertaken a more detailed analysis of optical parameters (wavelength λ, power P, and duration of laser trap exposure t) in cell trapping. Although it must be recognized that dissimilar cell systems and biological objects may vary in their response to different optical trapping parameters, a general guideline to the selection of optical parameters would be of significant value. In this paper we have examined the ability of cells to continue to proliferate and form clones after exposure of the nucleus to different laser trapping parameters.

MATERIALS AND METHODS

Cell culture

Chinese hamster (*Cricetulus griseus*) ovary (CHO) cells obtained from the American Type Culture Collection (CCL no. 61) were used in the experiments. The cells were maintained in GIBCO's minimum essential medium (MEM) with 0.025 mM phenol red and 10% (v/v) fetal bovine serum (Life Technologies, Grand Island, NY) and were regularly subcultured using 0.25% trypsin (Life Technologies). In preparation for an experiment, cells were grown in T-25 tissue culture flasks (Corning, Newark, CA) until they reached the desired confluence. The cells were then collected and injected into Rose chambers at a density of 3×10^3 cells/ml, 4–5 h before laser exposure.

Received for publication 7 April 1994 and in final form 15 November 1995.
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0006-3495/96/03/1529/05 $2.00

Laser microbeam instrumentation

Two optical trap systems were employed in this study: a Nd:YAG laser at 1064 nm (Quantronix 116, Smithtown, NY) and a titanium-sapphire laser tunable between 700 nm and 1000 nm (model 889; Coherent Inc., Palo Alto, CA) operating in the TEM_{00} mode. For both the Nd:YAG and the titanium-sapphire systems the lasers were directed into an upright Zeiss photomicroscope and subsequently focused by a Neofluar X100 phase-contrast objective with a numerical aperture of 1.3 (Carl Zeiss, Thornwood, NY) into a spot less than a micron in diameter with a typical TEM_{00} mode gaussian profile. A dichroic mirror deflected the laser beam into the photomicroscope while at the same time allowing visible light to pass to a video camera. The video image was recorded by a half-inch time lapse VCR (Panasonic Corp., Seaucus, NJ) and displayed on a monochrome monitor.

During the experiments, constant temperature at 37°C was maintained in the area of the microscope stage where the Rose chamber was placed by using an air-stream stage incubator (model ASI 400; Nicholson Precision Instruments, Bethesda, MD)

Calibration of laser power

To determine the power reaching the irradiated sample, the dual-objective transmittance-measuring technique of Misawa et al. (1991) was used. In this method, two identical and opposite-facing microscope objectives first focus and then recollimate the incident beam into an optical power meter. This method eliminates total internal reflection errors that are encountered in a direct objective-to-power meter measurement in air. In the dual objective method, the transmission through a single microscope objective is then the square root of the measured transmittance. In our experiment, the transmission through a single oil-immersion objective determined from the dual-objective method was 0.58. In comparison, a direct objective-to-power meter measurement in air gave a transmission of 0.33, which is 57% of the true value. Careful consideration should be given to the technique used in various published studies to measure laser irradiance at the focal point.

Laser microirradiation and cloning of single CHO cells

Isolated healthy single cells in interphase were chosen for all experiments. The position of the preselected cell was marked first by inscribing a small circle around it on the outside coverslip surface of the culture chamber using a Zeiss diamond objective marker. A second larger circle was drawn around the first diamond-cut circle using a permanent ink marker. The marker facilitated rapid visual relocation of the experimental single cell under the microscope during follow-up. The nucleus (about 7–10 μm in diameter) of the preselected cell was placed under the cross-hairs on the monitor screen, such that in each cell irradiation a random region of the nucleus was exposed to the trapping beam. The cross-hairs denoted the focal point of the optical trap. The laser trapping microirradiation was then initiated at this specific site for each laser wavelength. The diameter of the laser beam spot was ~0.6–0.7 μm. The laser trapping power in the objective focal spots was either 88 mW or 176 mW, corresponding to power densities of 2.6×10^7 W/cm^2 and 5.2×10^7 W/cm^2, respectively.

After laser microbeam irradiation, the Rose chamber was maintained in a CO_2 incubator at 37°C. The irradiated single cells were observed morphologically, and cell replication was continuously recorded until cell clones consisting of more than 50 healthy-looking cells were produced. This usually took 5–6 days.

Control experiments consisted of cloning of cells that were a) nonirradiated (i.e., exposed neither to laser trapping nor to normal microscope illumination); b) microscope-illuminated (exposed to normal microscope illumination for the same duration as cells that were used in trapping experiments); and c) cytoplasmic-irradiated (exposed in their cytoplasm to the same laser trapping parameters as the nuclear-exposed experimental cells; care was taken not to expose the perinuclear region of the cytoplasm because of the location of the centrosome is in that region).

In all laser exposure control and experimental studies, a minimum of 10 cells per parameter (i.e., per data point in all the figures) were exposed and individually assayed for clone formation. In the nonirradiated control series in which cells were exposed neither to laser nor to microscope illumination, 219 single cells were assayed for cloning. A total of 2471 cells were individually followed and assayed for clonal growth.

RESULTS

A 93% cloning efficiency was obtained in the control group of 219 cells exposed to neither laser nor light microscope illumination. One hundred percent of the 42 control cells exposed to microscope illumination alone grew into viable clones after exposure as long as 20 min. For cytoplasmic control laser exposures at 700 and 800 nm, the cloning efficiencies were 90% and 80%, respectively (Fig. 1).

Wavelength dependence of cloning efficiency for both laser focal spot powers (88 mW and 176 mW) is readily discernible in Figs. 2 and 3. The two figures depict similar results: 1) optimum cloning efficiency (60–100%) occurs at 950–990 nm; 2) minimum cloning efficiency (0–20%) occurs at 740–760 nm; 3) good cloning efficiency (50–90%) occurs at 800–850 nm for exposure durations of 3 min or less; and (4) poor cloning efficiency (less than 40%) occurs at 700 nm, 900 nm, and 1064 nm for exposure times of 5 min or longer.

It is clear from the data presented in Figs. 4 and 5 that at all wavelengths there is a decrease in cloning efficiency as a function of increasing the time of exposure to the trapping beam.

Table 1 presents cloning efficiency as a function of wavelength and energy density, ED (J/cm^2), where ED = power density $\times$ time. As expected, a general decrease in cloning efficiency is observed with increasing ED.

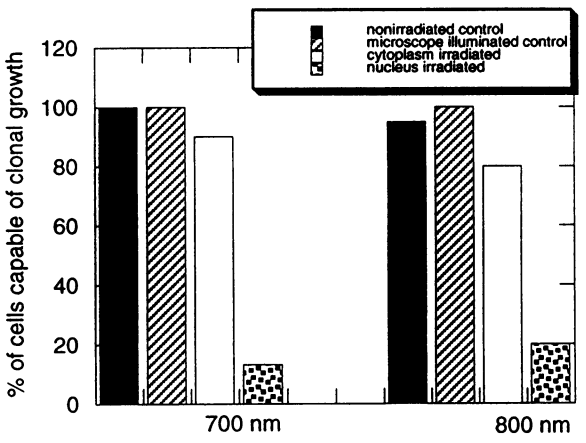

FIGURE 1 Comparison of cloning assay after laser microirradiation of nucleus and cytoplasm in CHO cells at 176 mW, 5-min exposure. Cloning efficiencies of nonirradiated cells and cells only exposed to microscope illumination are presented. The different experimental groups are nonirradiated control, microscope illuminated control, cytoplasm irradiated, and nucleus irradiated.

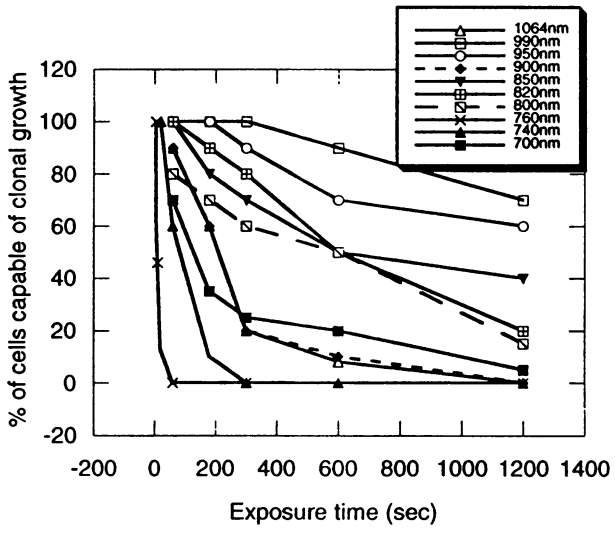

FIGURE 2 Wavelength dependence of cell cloning efficiency after optically trapping the nuclei in CHO cells using 88 mW. The following wavelengths were used (nm): 1064, 990, 950, 900, 850, 820, 800, 760, 740, and 700.

DISCUSSION

The data presented in this paper demonstrate that the ability of cells exposed to optical traps to divide and proliferate into viable clones is dependent upon several optical parameters: 1) wavelength, 2) power density, 3) energy density, and 4) duration of exposure. In the ideal laser trap there should be no absorption, thus no chemical or thermal events should be induced in the sample by the trapping beam. However, even with the least damaging trapping wavelength (950 nm) at the lower power (88 mW), the cloning efficiency decreased by 30% after 10 min in the trap (ED = 1.8×10^{10} J/cm^2). After 20 min in the trap it decreased by 40% (ED = 3.6×10^{10} J/cm^2) (Table 1).

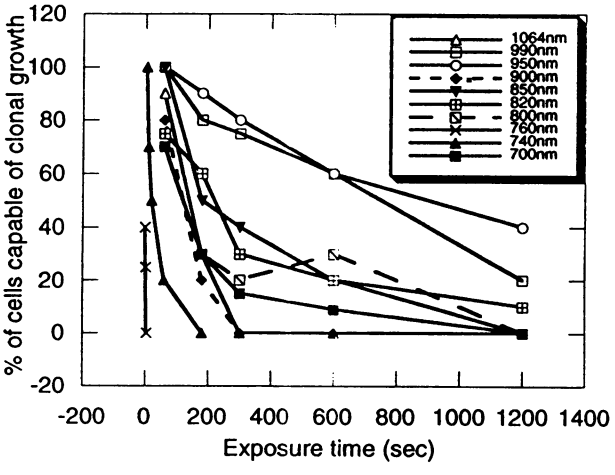

FIGURE 3 Wavelength dependence of cell cloning efficiency after optically trapping the nuclei in CHO cells using 176 mW. The following wavelengths were used (nm): 1064, 990, 950, 900, 850, 820, 800, 760, 740, and 700.

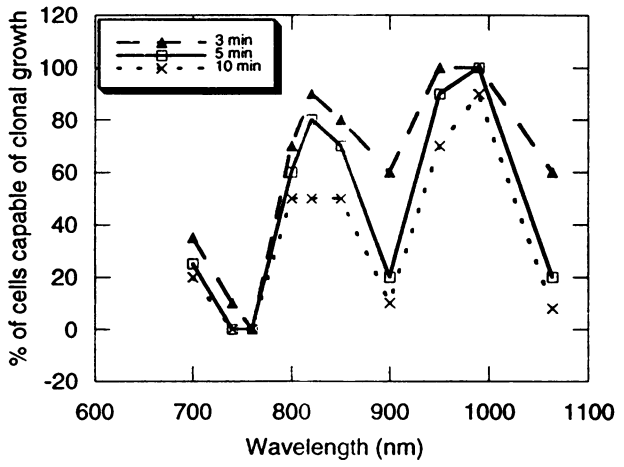

FIGURE 4 CHO cells cloning assay by wavelength and duration of exposure at 88 mW. Duration times were (min) 3, 5, and 10.

The wavelength-dependent effects of the optical trap on cell cloning efficiency reveal two regions of maximum cell survivability after exposure of the nucleus to typical laser powers used in optical trapping experiments: a) 950–990 nm and b) 800–850 nm (see Figs. 4 and 5). Although the former wavelength region appears superior, better than 50% cloning efficiency can be obtained with the latter wavelengths if the exposure time is 5 min or less. In addition, other trapping wavelengths may be permissible (see Figs. 2 and 3) if lower laser powers and/or exposure times are used. For example, at 88 mW the 1064 nm Nd:YAG trap yields a 60% cloning efficiency for a 3-min exposure. However, it would appear that laser traps employing 740–760 nm and the region around 900 nm should be avoided.

The duration of exposure to the laser trap has significant effects on the ability of the cell to survive and proliferate (Figs. 4 and 5). At the higher laser power (176 mW) after a 3-min exposure to the trap, cloning efficiency dropped to 60% or less, except for the 950 nm and 990 nm traps. It is

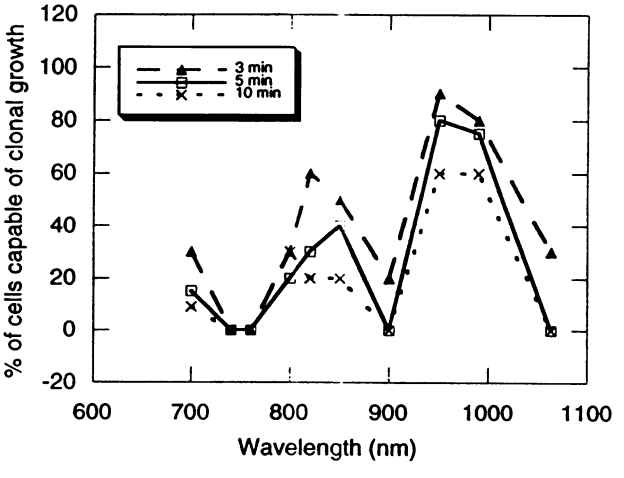

FIGURE 5 CHO cells cloning assay by wavelength and duration of exposure at 176 mW. Duration times were (min) 3, 5, and 10.

TABLE 1 Cloning efficiency in CHO cells as a function of energy density (power density × time) and wavelength

mW	88	176	88	88	176	88	176	88	176	176
min	1	1	3	5	3	10	5	20	10	20
*ED	18	36	54	90	108	180	180	360	360	720
λ (nm)										
700	‡70	70	35	25	30	20	15	5	9	0
740	60	20	10	0	0	0	0	0	0	0
760	0	0	0	0	0	0	0	0	0	0
800	80	70	70	60	30	50	20	15	30	0
820	100	75	90	80	60	50	30	20	20	10
850	100	100	80	70	50	50	40	40	20	0
900	90	80	60	20	20	10	0	0	0	0
950	100	100	100	90	90	70	80	60	60	40
990	100	100	100	100	80	90	75	70	60	20
1064	90	90	60	20	30	8	0	0	0	0

*Units: 10^8 J/cm^2. Does not take account of change of spot size with λ.
‡All values are a percentage of cells with clonal growth after exposure to the focused laser

interesting to note that for the more commonly used trapping wavelength, 1064 nm, cloning efficiency decreased to 30% in the 176 mW trap. Published studies routinely use trapping powers in the range of 50–200 mW in the laser focal spot. In addition, unless careful measurements are made on microscope objective transmission (see Materials and Methods), the power densities reported in the literature may, in fact, be considerably higher than what is stated.

Despite the concerns raised in the previous paragraph, it appears that except for 740–760 nm, the higher trapping power (176 mW) can be used without adverse effects if the duration of exposure is 3 min or less. Many biological experiments employ traps for only a few seconds to a couple of minutes. The cloning assays used in this study would suggest minimal effects to the cell and/or cellular processes using these laser parameters.

The fact that biological effects increase with laser exposure time or laser power density does imply an effect induced via absorption. Our earlier study demonstrating a temperature rise of 1.15°C/100 mW of laser power for CHO cells in a 1064 nm laser trap (Liu et al., 1995) supports this hypothesis. However, the inhibition of cell cloning at the wavelengths described in this paper and the previously described 740–760-nm wavelength dependency of induced chromosome bridges suggest that the mechanisms of alteration may be other than thermal. The power densities used in the studies reported here are 2–5 × 10^7 W/cm^2. This could result in an increase in temperature of 1–2°C. Even this level of temperature rise would be unlikely for wavelengths other than 1064 nm, considering the low water absorption in the spectral region of the laser wavelengths used (Palmer and Williams, 1974). Similarly, there are no known nuclear chromophores that absorb in the 700-1060-nm region of the spectrum. Although there are mitochondrial respiratory molecules that absorb near 700 nm, the control trapping experiments involving cytoplasmic exposure would seem to rule out an absorption effect by these molecules.

Another explanation for the observed inhibition of cell proliferation may be a multiphoton absorption process. We first suggested multiphoton-induced alteration of mitotic chromosomes in dividing cells (Calmettes and Berns, 1983). More recently, laser microscopes have been developed that specifically use two-photon absorption to induce focal plane-specific fluorescence (Denk et al., 1990). In both of these studies, short-pulse lasers were necessary to achieve the power densities needed to induce multiphoton events. However, recent observation of two-photon-induced fluorescence in motile sperm held in a 760-nm optical trap strongly suggests the possibility of two-photon-induced cell damage, also at lower power density (Konig et al., 1995).

Additional experiments using a variety of laser parameters and different cell cultures and synchronized cell populations are required to determine whether the hyperthermic or the two photon absorption mechanism is responsible for the observed wavelength dependence of cell cloning efficiency.

We thank Mr. Jeffrey Andrews and Mr. Glen Profeta for their assistance with the laser equipment and Dr. Chung-Ho Sun and Ms. Marie Hammer-Wilson for their suggestions concerning cell culture. We also thank Dr. William H. Wright for his valuable suggestions on this project and Mr. Yagang Liu for his assistance in the power measurements.

This work was supported by grants from the National Institutes of Health (RRO1192), the Office of Naval Research (ONR N00014-91-C-0134), the Department of Energy (DE-F03-91ER61227), and the Beckman Laser Institute Endowment.

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