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Micromanipulation of sperm by a laser generated optical trap*†

Yona Tadir, M.D.‡
William H. Wright, B.S.§
Omid Vafa§
Teri Ord‖
Ricardo H. Asch, M.D.‖
Michael W. Berns, Ph.D.§

Beckman Laser Institute and Medical Clinic, and the Department of Obstetrics and Gynecology, University of California, Irvine, Irvine, California

The mechanical force exerted on a microscopic particle by light is a result of momentum carried by the electromagnetic wave. A single beam gradient force trap[1] consists of a laser beam with a Gaussian intensity profile, focused to a spot smaller than the particle being trapped (Fig. 1). This trap confines the particle to a spot just below the focal point of the laser beam in the axial direction and centered in the beam in the transverse direction. The magnitude and direction of the net force on the particle is dominated by the refraction of the laser light through the object. The force generated by the light is greater than all other forces acting on the particle.

The manipulative capabilities of the optical trap have been demonstrated in experiments showing separation of individual bacteria from one sample to another. Viruses and red blood cells have also been trapped by focused laser beams.[2,3] Optical trapping has been applied in flow cytometry to selectively trap and manipulate individual cells with high-accuracy and minimal fluid flow.[4] Optical trapping in the infrared appears to cause little or no damage to the trapped object. Reproduction of bacteria, yeast cells,[1] and chromosome movement in animal *Potorous tridactylis* kidney cells following optical trapping has been observed.[5]

The rapid progress of modern reproductive technologies calls for the application of new micromanipulation procedures and the development of new tools. Recent advances in laser technology permit the application of laser microbeams to gametes. In this study, human sperm motility was studied using the force generated by radiation pressure of an infrared laser beam in order to evaluate the potential use of the optical trap as an experimental and operative tool.

MATERIALS AND METHODS

A single beam gradient force optical trap was employed in this study.[5] A Neodymium:Yttrium-Aluminium-Garnet (Nd:YAG) laser (Quantronix Model 116, Smithtown, NY) operating continuous wave (CW) in the transverse electromagnetic (TEM_{00}) mode at an infrared wavelength of 1.06 μm was directed into a Zeiss photomicroscope (Zeiss, Thornwood, NY) and focused into the field of view using a 40× Neofluar objective (Fig. 2). The power of the laser beam on the target was measured to be 1 Watt (±10%) in a focused spot with a diameter of 2 to 3 μm.

A motorized X-Y microscope stage was used to move the sperm confined in the optical trap. Remote real time viewing of the sperm in the trap was performed using a video camera (Dage-MTI, Series

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‡ Reprint request: Yona Tadir, M.D., Beckman Laser Institute and Medical Clinic, University of California, Irvine, 1002 Health Science Road, East, Irvine, California 92715.
§ Beckman Laser Institute and Medical Clinic, University of California, Irvine.
‖ Department of Obstetrics and Gynecology, University of California, Irvine.

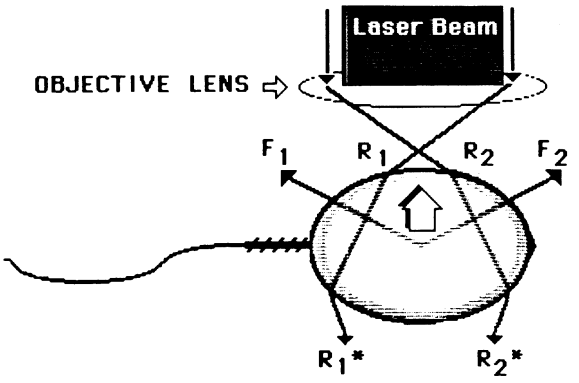

Figure 1 Ray diagram depicting the basic principles of the optical sperm trap. An infrared laser beam at a wavelength of 1.06 μm was directed through a 40× objective and focused above the sperm. A pair of incident and transmitted light rays are denoted by R and R*. The forces resulting from the refraction of the rays R1 and R2 are represented as F1 and F2, respectively. Transverse stability occurs when the sperm is centered in the trap.

68, Michigan City, IN) collinear with the path of the trapping beam. A dichroic mirror was used to separate the trapping beam from the image projected onto the video camera, and then recorded on a videotape for later analysis. The recorded images were analysed using an image processing system consisting of an Imaging Technology (Model 151, Woburn, MA) image processor connected to a IBM PC AT computer (IBM, Boca Raton, FL) acting as a host controller. The image processor was used to measure specific characteristics of sperm movement before and after exposure to the optical trap.

The exposure times in the trap were 15, 30, 45, 60, 90, and 120 seconds. A total of 514 sperm from 11 donors were trapped. Semen was washed and allowed to swim up in buffered hepes solution. This

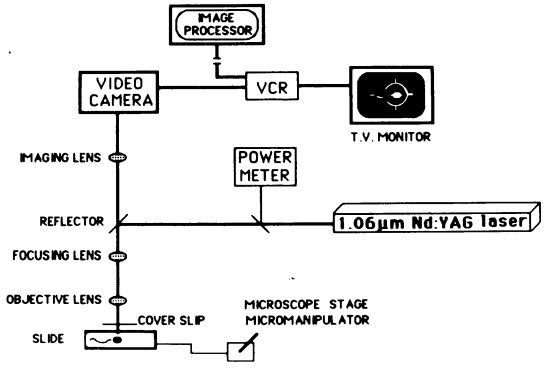

Figure 2 Schematic diagram of the optical trap. The microscope is represented by the objective lens and the focusing lens was mounted above the microscope.

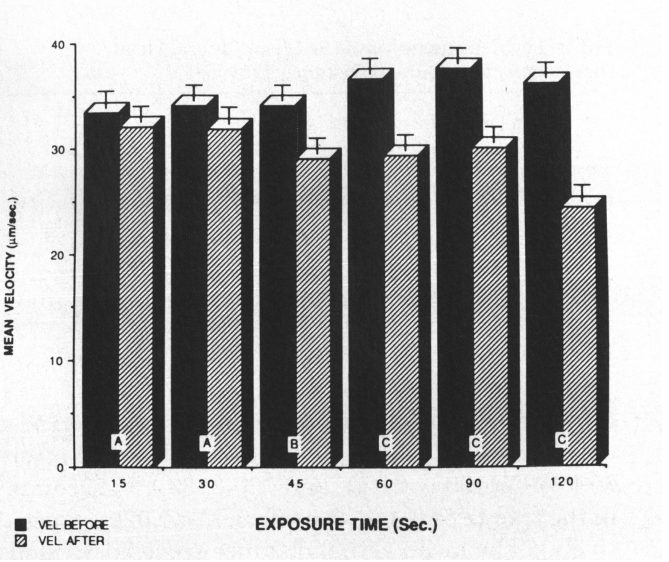

Figure 3 Changes in mean velocity (±SEM) of sperm exposed to the optical trap. Total number of sperm evaluated was 514. The number in each group varied from 63 to 97. (A: nonsignificant; B: $P < 0.0116$; C: $P < 0.0001$). Mean velocity before and after trapping is measured in μm/s.

was followed by a dilution in MEM (Gibco, Grand Island, NY) to obtain a density of 3 to 5 sperm in the visual field of the microscope. Experiments were performed on a glass slide under a number 1 cover slip, 4 to 8 hours after ejaculation. Morphologically normal sperm were selected. After different laser exposure time periods, motility characteristics such as linear velocity, actual distance traveled, maximum lateral head displacement and motility patterns (before, during, and after exposure) were recorded and analysed on a frame by frame basis (30 frames/s, 2 seconds before and 2 seconds after trapping).

RESULTS

The mean linear velocities of sperm before and after exposure to the laser trapping beam for various exposure times are summarized in Figure 3. There was no significant change in velocity for exposure times of 30 seconds or less. Sperm exposed to the trap for 45 seconds or longer had a statistically significant decrease in velocity when released. The sperm population was subdivided into two groups based on their initial velocity, slow (1 to 30 μm/s) and rapid (31 to 60 μm/s) motile. In the slow motile group, a significant (20%) increase ($P < 0.0124$) in linear velocity was observed for sperm after release from 15 seconds in the trap. After 30 and 45 seconds of exposure, there was no signifi-

Table 1 Measurements of the Mean Lateral Head Displacement and Actual Distance Traveled

Mean lateral head displacement			Actual distance travelled		
Before	After		Before	After	
Pixel distance[a]			*Pixel distance*[a]		
53 ± 3	44 ± 3	−17% ($P < 0.015$)	614 ± 31	566 ± 26	−7.8% ($P < 0.016$)

[a] Approximate distance per pixel is 0.05 μm.

cant change of mean velocity in this group. A gradual decrease in sperm velocity was observed after 60 (−19%), 90 (−21%), and 120 (−33.1%) seconds in the trap ($P < 0.091$, $P < 0.049$, $P < 0.021$, respectively). The mean actual distance traveled, as well as the mean lateral head displacement, decreased after all exposures (Table 1). This was similar (±4%) in all exposures. Motility for the rapid motile sperm was similar to the total population as illustrated in Figure 3.

Sperm motility characteristics were defined according to four different patterns.[6] From the analyzed sperm population before optical trapping, 39% were straight forward progressing, 58% possessed a zig-zag pattern, and the remainder were classified as bending, or circular patterns. The pattern changes after short exposures (<30 seconds) were minimal for the initially straight progressing sperm. Of the zig-zag moving sperm, 40% changed to straight forward progression after release from the trap. There was a gradual increase in the pattern change in straight progressing sperm as well as for the sperm with zig-zag pattern after longer exposures (>45 seconds).

Manipulation of up to three sperm at the same time in the trap was possible. Reduction of the laser power in the range of 50% to 95% from the highest setting lowered the number of sperm that could be caught and manipulated in the trap. There was a clear power threshold that allows the sperm to be spontaneously released.

DISCUSSION

The optical trap may be used as a micromanipulator, permitting sperm traction in vitro. The present system's trapping capability is limited to a maximum of three sperm in the trap. We have demonstrated that laser light can be used to manipulate sperm which may be applicable to experimental studies on sperm physiology. It is also possible that optical trapping may be incorporated as an active element into the available computerized semen analysis systems which currently do not employ manipulative techniques.

From the analysis of the mean linear sperm velocity before and after trapping, we conclude that sperm are not significantly affected by the trap after short periods of exposure (up to 30 seconds). Longer exposures caused a decrease in sperm velocity and altered the motility pattern, possibly by absorption of the laser beam and subsequent heating. However, it is not known if the magnitude of this reduction is of clinical relevance. It may be possible to trap sperm at a lower laser power level than that used in this study. The observed increase in velocity of slow motile sperm (<30 μm/s) after short exposures may be a result of the decrease in the amplitude, of the lateral head displacement. This decrease in amplitude was possibly caused by the optical trap, resulting in a straightening of the sperm tail. After release from the trap, the majority of the sperm that demonstrated an altered pattern had a tendency to become straight forward progressing. Sperm with different motility patterns that were trapped for longer periods (>120 seconds) revealed a sequence of changes as they approached immotility: from straight progression to zig-zag movements; from zig-zag to bending; from bending to circular; followed by irregular oscillations leading to immotility. These observations were determined by reviewing the video recordings as well as by computerized image processing.

Ashkin and Dziedzic[2] indicated that exposure of live cells to the laser with minimal tapping intensity does not result in visible cell damage. However, in view of our observed effects on motility, additional ultrastructural and chromosomal analysis must be performed to determine any possible detrimental effects before the optical trap is used clinically to manipulate sperm.

We have demonstrated that optical trapping and micromanipulation of sperm using a low power laser beam is technically feasible. We are currently measuring the force generated by sperm to induce motility. This is being done by gradually decreasing the trapping power levels to allow spontaneous sperm release. These values are then correlated to velocity and motility patterns. Measurements of sperm force before and after the introduction of drugs that are known to affect sperm motility may provide a more accurate determination on the possible effects of such chemicals. From these experi-

ments, the optical trapping of sperm may be developed into a new micromanipulation technique.

SUMMARY

The force generated by the radiation pressure of a low power laser beam induces an optical trap which may be used to manipulate sperm. We studied the effect of the optical trap on sperm motility. A Nd:YAG laser beam was coupled to a conventional microscope and focused into the viewing plane by the objective lens. Sperm were caught in the trap and manipulated by a joy stick controlled motorized stage. After different exposure periods, the velocity and patterns were analysed by a computerized image processor. There were minor changes in sperm velocity when exposed to the trap for 30 seconds or less. A gradual decrease in the mean linear velocity was observed after 45 seconds of exposure. This optical micromanipulator may also be useful for studying the force generated by a single spermatozoa and evaluating the influence of drugs on motility.

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