Optical determination of motility forces in human spermatozoa with laser tweezers

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ABSTRACT

Laser tweezers may act as optical force transducers. We report on the determination of intrinsic motility forces of human spermatozoa by employing an 800 nm optical trap. The cellular forces were calculated from calibrated trapping forces. The determination of trapping forces based on a hydrodynamic model for ellipsoidal specimens, the measurement of the minimum laser power required to confine a single cell in the trap, and the calculation of viscous forces during the movement of optically trapped sperm heads through a laminar fluid. A mean motility force of 44±24 pN was calculated for spermatozoa of healthy donors.

KEYWORDS: spermatozoa, laser tweezers, optical trap, motility force

1. INTRODUCTION

In industrial countries, the proportion of unwanted sterile couples is about 10% (1). One reason is poor sperm quality (reduced spermatozoa concentration/reduced motility). In vitro fertilization (IVF) may help these couples who want to have their own children. In the case of male infertility, IVF can imply mechanical micromanipulation (micropipettes) of spermatozoa as well as optical micromanipulation by laser tweezers (optical traps)[2-4]. To improve understanding of male infertility and of assisted IVF the characterization of the motility forces of human spermatozoa in absolute terms would be helpful.

One way to yield information on the cellular motility forces is by employing optical forces of laser tweezers. Optical forces arise during the interaction of a highly focused near infrared (NIR) laser beam with cells or cell organelles which are determined primarily by intracellular beam refraction. Such optical forces are used to create stable 3D optical traps. The net force F, also called trapping force, may be sufficient to confine even motile single cells in the focal volume of the objective (single beam gradient force optical trap)[2]. The trapping force can be estimated by:

$$F = Q\frac{P}{c} \qquad (1)$$

where c is the velocity of light in medium, P the incident laser power at the sample (*in situ* power), and Q the dimensionless trapping efficiency which depends on the sample as well as on the beam properties. The one to one correspondence between force and *in situ* power enables the use of laser tweezers in force determination. Such force transducers were used, for example, to determine the

compliance of the rotary motor located in bacteria flagellum[5], forces for organelle transport[6], and forces exerted by kinesin[7,8].

In order to obtain absolute numbers of trapping forces, calibration has to be done for the special experimental set-up. In the case of spherical samples, calibration can be performed by moving the optically trapped sample at a certain *in situ* power through a laminar fluid and by the measurement of the maximum (drop off) trapping velocity v_{max} where the sample "escapes" from the trap. At that speed, F is equal the (viscous) drag force F_{drag} which can be calculated from the Stokes equation by :

$$F = 6\pi r \mu v_{max} \qquad (2)$$

where r and μ are, respectively, the radius of the spherical sample and the dynamic viscosity of the medium. However, non-spherical specimens, such as human spermatozoa, require the impact on hydrodynamic models which consider the geometry of the trapped sample.

2. MATERIALS AND METHODS

The 800 nm beam of a cw Ti:Sapphire ring laser (899-01, Coherent, CA) was expanded to fill the back aperture of a 100x Zeiss Neofluar brightfield oil immersion objective (NA=1.3) of an inverted Zeiss microscope. The diffraction-limited, highly focused microbeam served as laser tweezers. The tweezers were used to determine longitudinal forces (perpendicular to the optical axis).
Additionally, frequency-doubled Q-switched Nd:YAG laser radiation (532 nm, 4-6 ns, Surelite 1, Continuum) was coupled into the microscope and used for flagellum removal ("laser scissors"). The real *in situ* power (power at the sample) was determined using a sandwich system containing a microchamber and two identical Zeiss brightfield objectives as described elsewhere[9].

Polystyrene microspheres with the diameter $2r = 3.004 \pm 0.029$ μm and the refractive index $n = 1.57$ (VIS + NIR) were used as a model for spherical specimens.
Semen specimens were obtained within three hours following ejaculation from donors with normal semen parameters according to the WHO guidelines. Semen was layered on a discontinuous isotonic percoll gradient consisting of three 1 ml layers of 95%, 70%, and 50%. After centrifugation, the bottom layer was removed, washed with HEPES buffered fresh human tubal fluid (HTF, Irvine Scientific) and centrifuged. The pellet containing sperm was then diluted in HEPES buffered isotonic saline solution containing 1% human serum albumin (HSA). Spermatozoa were injected into sterile microchambers. Measurements on a total number of 142 sperm cells were carried out at 29°C room temperature.

3. RESULTS

Experiments with Microspheres

Single microspheres were trapped 5 μm above glass bottom (coverslip) of the microchamber by 800nm-laser tweezers. The motorized stage was moved over a 2 mm distance with variable velocities (multiples of 0.48 mm/s, acceleration: 9 cm/s^2). The maximum trapping velocity v_{max} was determined where the trap was still able to confine the microsphere. The figure exhibits the v_{max} linear dependence on the *in situ* laser power P with $v_{max} = P \cdot 0.0052$ $cm\ s^{-1}\ mW^{-1}$.

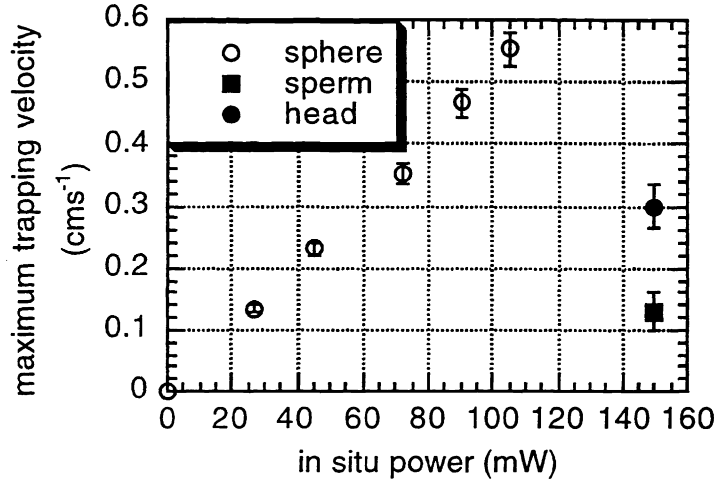

Figure Maximum trapping velocity v_{max} of a 3 μm microsphere in an 800nm optical trap vs. *in situ* laser power, and values for isolated sperm heads and paralyzed spermatozoa

For viscous force calculation, the distance h between sample and coverslip has to be considered[10] by the correction factor β:

$$\beta = 1(1-9/16(r/h) + 1/8(r/h)^3 - 45/256(r/h)^4 - 1/16(r/h)^5) = 1.2 \qquad (3)$$

with r/h = 1.5/5 = 0.3. According to formula (2) and (3) we can calculate the viscous force at 100 mW *in situ* power ($v_{max} = 0.52 \pm 0.04$ cm/s):

$$F\,(100\text{ mW}) = 6\pi r \mu v_{max} = 143 \pm 11\text{ pN}$$

with μ= 0.81 cP (water, 29°C). This force, calculated for the maximum trapping velocity, is equal to the trapping force. According formula (1), the trapping efficiency is therefore:

$$Q = 0.32 \pm 0.02\ (c_{medium} = 2.25 \cdot 10^8\text{ m/s}).$$

3.2. Cell Experiments

A total of 122 cells with initial movement in the plane perpendicular to the optical axis (x,y-plane) were confined at h = 5.0±0.5 μm with an 800 nm microbeam of 150 mW power. The error of 0.5 μm considers the difference between stage moving and moving of the trapped cell due to the different refractive indices of medium and immersion oil[11].

At first, the mean minimum trapping power of 82±38 mW required to hold the sperm in this special optical trap was determined by step-wise laser power reduction. In general, trapped sperm kept the initial direction of movement in the x,y-plane prior to trapping after escape from the trap ("memory effect").

In a next step v_{max} of sperm heads was determined at 150 mW trapping power. For that purpose the tail and midpiece of ten cells were excised using the 532nm pulsed microbeam. We found a mean value of 0.30±0.04 cm/s. In contrast, paralyzed sperm showed decreased values indicating the influence of the midpiece and the flagellum (see Figure).

The normal human sperm head has a geometry that can be approximated with an ellipsoid. For an ellipsoidal shape, the drag force on the head can be calculated[12] with:

$$F_{drag} = 6\pi R_{eq} \mu v_{max} \qquad (4)$$

where R_{eq} is a parameter given by the half axis lengths. Typical half axis values for the human sperm head are a= 2.5 μm, b= 1.0 μm and c= 1.5 μm.

We developed formula (5) for R_{eq} calculation in the case $1<a/b<5$ and b=c as described elsewhere[9]:

$$R_{eq} = 0.1963a + 0.8037b \qquad (5)$$

With the approximation of an ellipsoid with half axes of same length by taking the average of b and c of a typical human sperm: (1.5 μm+1.0 μm)/2=1.25 μm, R_{eq} yields 1.50 μm. This value is the same as the radius of the microspheres described above.

With equation (2), F_{drag} yields for 150 mW *in situ* power:

$$F_{drag} = \beta 6\pi\mu \text{ x } 1.5 \text{ μm x } (0.30\pm0.04) \text{ cms}^{-1} = (82\pm10) \text{ pN}$$

With formula (1) and $F = F_{drag}$ we obtain finally a Q-value for sperm heads of

$$Q_h = 0.12\pm0.02.$$

With the assumption of linear swim motion, the intrinsic motility forces of motile human spermatozoa can be calculated by use of the Q-value for human sperm heads and the measured minimum trapping power of 82±38 mW. Because the trapping beam interacts only with the sperm head[13], the Q-value for intact sperm will be also Q_h. The mean intrinsic force F_{in} of motile human spermatozoa in our sample can therefore according formula (1) and (9) determined to be:

$$F_{in} = 1/c \text{ x } (0.12\pm0.02) \text{ x } (82\pm38) \text{ mW} = 44 \pm 24 \text{ pN}.$$

In recent experiments[14,15] with epididymal sperm and frozen-thawed sperm with 800nm optical traps performed at the Beckman Laser Institute, minimum trapping power values were determined.

Assuming similar trap properties, the knowledge of Q (800 nm) for human spermatozoa enables the calculation of the mean motility forces. The calculated values from these high-cell-number studies are in excellent agreement with our 44 pN value:

epididymal spermatozoa:	18 pN (n= 1070)
ejaculated and frozen-thawed sperm:	45 pN (n= 970).

4. DISCUSSION

Our model of intrinsic force calculation bases on the assumption of linear cell motion and neglects the presence of forces in z-direction. As demonstrated by Lighthill[16], eukaryotic flagellar motions are more complicated and require consideration of undulations (helical movements) and sinusoidal transverse forces, respectively. However, we found that 92% of trapped sperm escaped in the initial direction prior to trapping ("memory effect"). Only 8% of the cells (excluded in calculations) lost memory and escaped in random direction.

The differences in the Q-values of microspheres and spermatozoa in spite of the same equivalent radius reflects the influence of the refraction index.

Baltz et al.[17] determined sperm motility forces mechanically using a suction micropipette. Because of difficulties in sperm micromanipulation, only 15 out of 10,000 investigated motile spermatozoa could be used in force calculations. The authors calculated values of thrust-derived and torque-derived forces of 16 and 190 pN. Bonder et al.[18] reported on forces of sea urchin and human sperm of 10-60 pN using optical tweezers. However, they did not consider the non-spherical shape of human sperm.

Our results demonstrate the potential of optical traps as non-contact sterile tools in force measurements even for non-spherical samples, such as ellipoidal specimens (human sperm heads).

5. REFERENCES

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