Using a fixed beam with a fundamental Gaussian mode, a gradient force optical trap was used to translate a single cell using a carboxylated polystyrene bead as a handle. Currently, the force with which the optical trap can manipulate an individual cell alone is very weak. In fact, the inadequate nature of the optical trap on living cells has prevented this technique from being useful in most research involving single cells. However, under the experimental setup used here, the trapping force of a particular neuronal cell line, CATH.a, was increased by over 270% by using carboxylated polystyrene beads as a handle. As a result, optical trapping is now a feasible technique for non-invasively translating individual cells during research.

**Introduction**

The development of gradient force optical traps (optical tweezers) has made the non-invasive manipulation of biological specimens possible with a single laser beam [1, 2]. With this technique, microscopic biological specimens can be individually manipulated, a capability invaluable for single cell control and resulting single cell experimentation. The single laser beam traps the biological specimen by imparting angular momentum on the focused biological specimen and thereby suspending it in place. Therefore, in moving the microscope stage, the biological specimen is fixed. This non-invasive technique is essential for many research projects including the publicized lab-on-a-chip.

In many experimental settings, the force with which optical tweezers trap specimens is insufficient. Thus, many studies have attempted to determine how to increase the trapping force. The force is defined by the simple relationship

\[
F = \frac{Qn_m P}{c}
\]

where \(Q\) is a dimensionless efficiency, \(n_m\) is the index of refraction of the suspending medium, \(c\) is the speed of light, and \(P\) is the incident laser power measured at the specimen [3]. Because biological specimens are usually suspended in aqueous media, the dependence of \(F\) on \(n_m\) can rarely be exploited to achieve higher trapping forces [4]. Increasing the laser intensity is possible, but only over a limited range due to the possibility of optical damage [5, 6]. \(Q\) itself is therefore the main determinant of trapping force. It depends on the numerical aperture (NA), laser wavelength, light polarization state, laser mode structure, relative index of refraction, and geometry of the particle [3]. This study exploits the direct dependence of \(Q\) on the difference in refractive indices between the trapped specimen and the suspending medium. By increasing the refractive index of the trapped species while leaving the refractive index of the suspending medium constant, the forces of trapping increases because \(Q\) increases.

Theoretically, trapping force is represented by Equation 1. However, trapping forces are experimentally determined relative to viscous drag exerted by the suspending medium. By measuring the maximum velocity after which the trapped species is lost from the optical tweezers, it is possible to determine the force by the following viscosity equation:

\[
F = \beta v
\]

where \(\beta\) is the drag coefficient and \(v\) is the particle velocity [3]. \(\beta\) is typically given by Stokes' Law:

\[
\beta = 6\pi n a
\]

where \(n\) is the fluid viscosity and \(a\) is the radius of the trapped sphere. Using the measured maximum velocity and the radius of the specimen, the optical trapping force was calculated using Equations 2 and 3.

It has been shown that microscopic particles can be attached to biological
specimens and used as handles in the rotational manipulation of biological specimen [7]. However, this study utilizes microscopic particles to enhance translational manipulation of biological specimen. Carboxylated polystyrene microparticles were selected as handles because of their large refractive index and their low cost. In this experiment, the polystyrene microparticles were trapped and made to adhere to CATH.a cells. The resulting complex is called a CATH.a/polystyrene bead complex. Once the complex was created, the trapping force on it was compared to that of the CATH.a cells and carboxylated polystyrene microparticles alone in order to determine the efficacy of using polystyrene beads as handles.

Materials and Methods

Sample Preparation

CATH.a neuronal cells were used as the biological specimen. A culture of CATH.a cells was grown in an isotonic medium (containing inorganic salts, vitamins, organic acids, and glucose). Prior to use, non-adherent CATH.a cells were removed from the culture and washed twice with Dulbecco’s phosphate-buffered saline (Gibco) and then suspended in the same buffer solution. Carboxylated polystyrene microparticles were obtained from Bangs Laboratories (Fishers, Indiana). The spherical particles were 2.60µm in diameter with a refractive index between 1.57 and 1.60. Plastic coverslips were incubated in a 1% Bovine Serum Albumin (BSA: Sigma 33035) solution for 1 hour. The BSA coated coverslips were used to limit cell to coverslip adhesion. The proper concentrations of the carboxylated polystyrene beads and the CATH.a cells were mixed to allow for the formation of predominately one-to-one coupling. The resulting solution was placed on the plastic BSA coated coverslips. Solutions with similar concentrations of only beads and only CATH.a cells were also used as controls.

Optical Tweezers and Force Measurements

The trapping force of CATH.a cells, polystyrene beads, and CATH.a/polystyrene bead complexes was measured. The particles were trapped by the laser beam and the critical velocity at which they were lost was measured. This measurement was obtained by using the automated microscope stage (Prior H107X) to control the acceleration, speed, and displacement of the stage movements quantitatively. In order to utilize the automated microscope stage, a serial-communicating driver was written using National Instruments LabVIEW 6i. The CATH.a/polystyrene bead complexes were formed in two steps: (1) a bead was trapped and moved next to a resting, free cell, (2) the cell adhered spontaneously to the bead. The cell could then be manipulated using the polystyrene bead as a handle.

Figure 1 shows the experimental setup used for studying the optical trapping force on biological specimens. A continuous-wave Nd:YAG was used as a light source. The laser was operated in the near infrared with a wavelength of 1064nm. A current of 4.5 amps was applied to the laser using the Uniphase PS006 controller. The laser light was expanded with a telescope, reflected by a dichroic beam splitter (442nm/1064nm), and focused into the specimen plane through a high N.A. objective lens (Zeiss Achromplan 100x) in an inverted microscope (Zeiss Axiovert 135). The specimen was placed close to the focus of the objective and was backlit with an incandescent lamp. The specimen was monitored through the same objective lens as mentioned above and the dichroic beam splitter using a charged-coupled device (CCD) camera. Digital pictures were collected using a frame grabber on a personal computer (Scion Image).

Results and Discussion

The optical trapping force data for CATH.a cells, polystyrene beads, and CATH.a/polystyrene bead complexes is shown in Figure 2. First, the average trapping force on a single CATH.a cell was 17pN ± 1pN (standard error), measured for eight CATH.a cells. This measurement serves as a baseline. Since the trapped specimens were not spherical, an average radius was used during calculations.

Second, the trapping force on a single polystyrene bead was 70pN ± 1pN (standard error), measured for eight polystyrene beads. This measurement serves as a standard comparison point. These beads were assumed to have a radius of 1.30µm, as specified by Bangs.
Laboratories. Finally, the trapping force on a CATH.a/polystyrene bead complex was 63pN ± 2pN (standard error), measured for six CATH.a/polystyrene bead complexes. The radii of the six complexes were measured independently. Figure 3 shows a sample cell/bead complex.

Although adhesion between the carboxylated polystyrene beads and the CATH.a cells was observed, the mechanism for this is unclear. At physiological pH, the carboxylated polystyrene beads have a negative surface charge. However, the phospholipid bilayer that makes up the majority of the membrane of the CATH.a cell is also negatively charged. Therefore, the mechanism of bead adherence upon CATH.a cells is not completely understood. Perhaps ionic interactions between positively charged transmembrane glycoproteins and the negatively charged beads accounts for the adherence.

The experiment has demonstrated that using carboxylated polystyrene microparticles as handles increases the optical trapping strength of CATH.a cells by 270%. It is important to note that Equations 2 and 3 predicted that the CATH.a/polystyrene bead complexes would exhibit a lower optical trapping strength than the polystyrene beads alone since the complex must have a larger radius. The above experimental results agree with this theoretical analysis. Moreover, the technique can be advanced by using beads made of materials with a larger refractive index. This would further increase the optical trapping strength as given by Equation 1.

The observed increase in trapping force makes optical trapping a feasible manipulative technique in microfluidic devices. In particular, using a polystyrene bead as a handle for optical trapping allows the manipulation of a single cell through devices such as the heavily researched lab-on-a-chip. In its theoretical form, the lab-on-a-chip is a fully automated microscopic chip that conducts certain reactions and the resulting analysis on individual cells. Using polystyrene beads as handles, optical tweezers can now be used to guide an individual cell through the chip.
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