Optoelectronic Tweezers for Cell Manipulation and Sorting

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Abstract: Optoelectronic tweezers is used to spatial pattern cells of varying types. OET manipulation is also used to distinguish live sperm from dead sperm based on sperm velocity in response to OET actuation.

1. Introduction

In biological research, microscale manipulation enables the study of single-cell behavior, as well as the sorting of specific target cells from a mixed population. Several types of forces can be used to manipulate microscale objects, including optical and electrical forces [1, 2]. However, optical traps can cause optically-induced cellular damage [3], while electrical traps have difficulty in manipulating *specific* individual cells. It would be ideal to combine the more benign electrical manipulation with more flexible optical control.

Our research group has developed a device that combines the advantages of optical and electrical manipulation, called optoelectronic tweezers (OET) [4]. Light patterns are used to create manipulation patterns and particle traps in an amorphous-silicon-based semiconductor device. The optical patterns create dielectrophoretic force in the OET device, via light-induced dielectrophoresis (DEP) [4]. Thus, OET does not directly use optical energy for trapping, allowing the use of much lower light intensities than direct optical manipulation. These low optical intensities can be achieved by a computer projector or an LED, allowing the creation of complex manipulation patterns. Furthermore, unlike electrical traps, OET is capable of trapping a *specific* single cell from a larger population.

Here we present recent work in using OET for cell manipulation and patterning, and for sorting live sperm from dead sperm with the goal of improving assisted reproductive technology techniques.

2. Cell Patterning

The OET device can be used to pattern cells in specific configurations and locations for studying cell-cell interactions or to precisely position cells before culturing. This is demonstrated by the arrangement of Jurkat cells into a cell array (Fig. 1). The randomly distributed cells are rearranged by OET manipulation into an organized individually addressable cell array. The optical manipulation patterns are created under direct user control using a custom-designed software interface, and are visible as the bright spots in the images.

Multiple cell types can also be simultaneously trapped and transported using OET. This is demonstrated with live fluorescent-labeled Jurkat cells and unlabeled HeLa cells (Fig. 2). Once the fluorescing Jurkat cells and non-fluorescing HeLa cells are identified, the cells are subsequently trapped and transported using OET. Thus, the original random distribution of the two cell types can be organized into segregated patterns for further single-cell study (Fig. 2d).

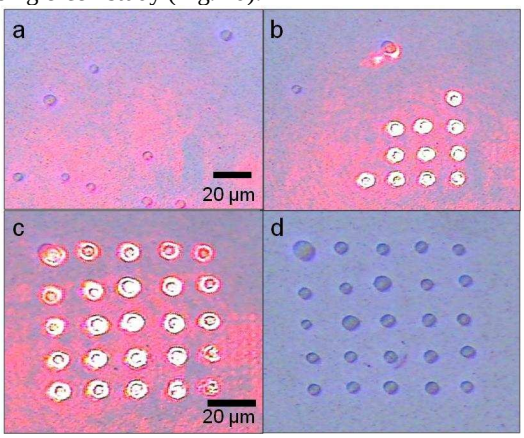

Fig.1. Formation of an array of live Jurkat cells using OET. (a, b) The randomly distributed cells are trapped and transported using OET manipulation patterns. (c) A 5 x 5 individually addressable cell array is formed. (d) The same array, with the OET manipulation pattern temporarily shut off for cell imaging clarity.

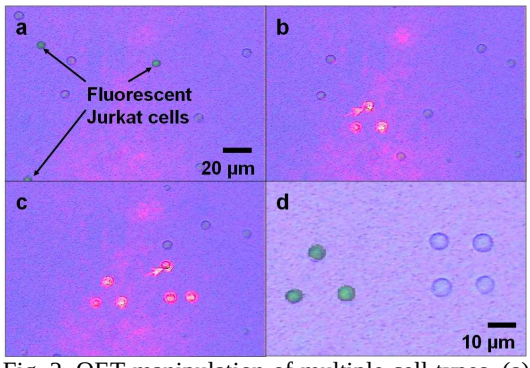

Fig. 2. OET manipulation of multiple cell types. (a) The fluorescent-labeled Jurkat cells are distinguished from the unlabeled HeLa cells (composite image). (b) The Jurkat cells are arranged in a triangular pattern. (c) The HeLa cells are arranged in a square pattern. (d) Fluorescent imaging verifies that the cell types are segregated (composite image).

3. Cell Sorting

One treatment that is available for infertility patients with low sperm counts and/or motility is intracytoplasmic sperm injection (ICSI). A major concern of the ICSI procedure is that natural sperm selection mechanisms are bypassed, making it imperative that healthy viable sperm are used. The selection of viable sperm based upon sperm motility is ineffective on samples with reduced sperm motility [5]. Nevertheless, even in samples with no motile sperm, up to 50% of the non-motile sperm remain viable [6]. In these instances, it is desirable to analyze the viability of the available non-motile sperm.

Current sperm viability assays, such as the Trypan Blue dye exclusion test and eosin-nigrosin staining, are not used for ICSI as they render the tested sperm unusable. The hypo-osmotic swelling test is potentially non-damaging, but the collection of viable sperm following testing is still performed manually [6]. As a result, sperm for typical ICSI procedures are still subjectively selected by a technician based on morphology.

Optoelectronic tweezers can provide a method of distinguishing between live and dead non-motile sperm. In addition, viable sperm that is identified using OET can be collected in parallel, and transported off-chip for use in ICSI procedures.

The dielectrophoretic force exerted by OET is a function of the frequency-dependent electrical properties of the cells under manipulation. As different cell types exhibit dissimilar electrical properties, DEP can be used to sort between cell types, or even between widely varying cells of the same type [7, 8]. We use this capability to selectively distinguish live non-motile sperm from dead sperm due to a marked difference in electric polarizability for live and dead cells. Thus, for an electric field at 100 kHz, live sperm will be attracted to the optical patterns, while dead sperm will be repelled.

Fresh ejaculate specimens from 6 healthy males were evaluated using OET. In order to determine the viability of non-motile cells, the samples were mixed in a 1:1 volume ratio with 0.4% Trypan Blue dye in DI water, and incubated at room temperature for 3 minutes. The sperm/Trypan mixture was then diluted approximately 100 times by adding a low-conductivity isotonic solution.

OET-induced velocity measurements were performed on a total of 300 individual sperm from the 6 donors. All (100%) sperm experiencing an attractive OET response were Trypan Blue negative (N = 150). The Trypan-Blue-positive sperm demonstrated either no response (54%) or a weak repulsive response (46%) to OET. A few Trypan-Blue-negative sperm (15%) demonstrated no response to OET actuation, suggesting that these sperm are also dead.

The average velocity of live non-motile sperm in the OET device is 8.0 ± 3.9 µm/s, averaged over 150 cells from 6 separate donors (Fig. 3). The average velocity of dead sperm is -1.0 ± 1.2 µm/s with the negative value indicating a repulsive response to OET force. The dead Trypan-Blue-positive sperm exhibited some variability in their OET response, exhibiting either weak negative OET response (54%) or no response to the OET pattern (46%). However, no Trypan-Blue-positive sperm exhibited a positive OET response. Thus, these results show a clear separation of the cell subpopulations.

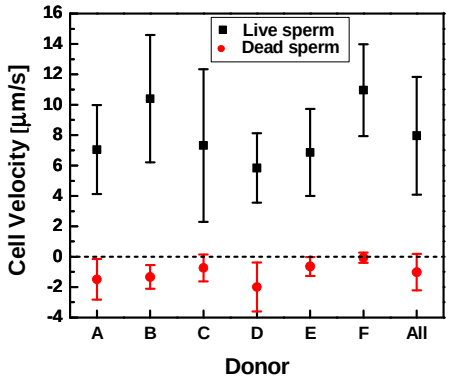

Fig. 3. OET-induced velocities of live non-motile sperm and dead sperm. The error bars indicate the standard deviation. "All" refers to sperm velocities averaged across all 6 donors.

4. Conclusions

The use of OET for optically controlled cell patterning and sorting was demonstrated. Live Jurkat and HeLa cells were controllably patterned under direct user control. Live non-motile sperm is shown to have an attractive response to OET at 8.0 ± 3.9 µm/s, while dead sperm are repelled at -1.0 ± 1.2 µm/s. This clear distinction in sperm response will allow OET selection of sperm for use in assisted reproductive procedures.

5. References

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