

Nondestructive Manipulation of Single Live Plant Cell by Laser Induced Micro Shockwave

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SUMMARY

We successfully demonstrated to manipulate single live plant cells using a laser induced micro shockwave, which was generated by focusing femtosecond laser on culture medium under microscope. Firstly, properties of shockwave were estimated by microparticles floating in a culture medium. It was suggested that the shockwave has an enough large force to easily handle μm -size biological cells, which is much larger than that of a conventional laser trapping. Secondly, to confirm the interaction between the shockwave and live cells, the force of shockwave was applied to separate two daughter cells of fission yeast. Finally, nondestructive isolation of sperm cell from pollen tube of buckwheat was presented.

Keywords: Femtosecond laser, microscope, nondestructive manipulation, yeast cell, sperm cell, buckwheat

INTRODUCTION

An individual cell manipulation using laser and microscope has attracted significant attention as a novel biological technique. Some kind of single cell manipulation for both plant and animal cells have been demonstrated by using laser trapping and laser ablation (1-5). Recently, infrared laser as a source of trapping laser has become user-friendly machine with improving the device technology, so that even operators who are not familiar with laser and microscope can perform laser manipulation. However, usage of the laser trapping is limited, because the optical pressure is not so strong (6). Indeed, when laser trapping was used in the cell manipulation, it is impossible to isolate single cell from tissue or cell culture plate. To overcome this problem, we have proposed a new method using nonlinear effects of femtosecond laser (7).

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When an intense femtosecond laser is focused on a transparent organic/biological material, various nonlinear photophysical and photochemical dynamic processes, such as, shockwave, cavitation bubble, plasma, and so on, are induced as a result of high efficient multiphoton absorption, mutual interactions between excited states, and/or ionization (8-12). Especially, the force of the shockwave is considered to become much larger than that of the conventional laser trapping. Hence, the shockwave has a potential to realize a single cell manipulation which is impossible only by conventional cell manipulation, for example illustration in Fig. 1, where it is supported that single cell manipulation in tissue is performed by combining the shockwave manipulation with laser trapping.

In this paper, single cell manipulation using the shockwave was demonstrated. The shockwave was generated by focusing an intense femtosecond laser to a culture medium. Firstly properties of the shockwave were estimated by microparticles floating into the culture medium (Section 1). It suggested an enough large force induced by shockwave to easily handle μm -size biological cells. Secondly, as a simple model, to confirm the interaction between the shockwave and live cells, we applied the shockwave force to separate two daughter cells of fission yeast (Section 2). Finally, nondestructive isolation of sperm cell in buckwheat from pollen tube was successfully demonstrated (Section 3). This paper shows that the femtosecond laser induced shockwave is effective for nondestructive manipulation of live cells.

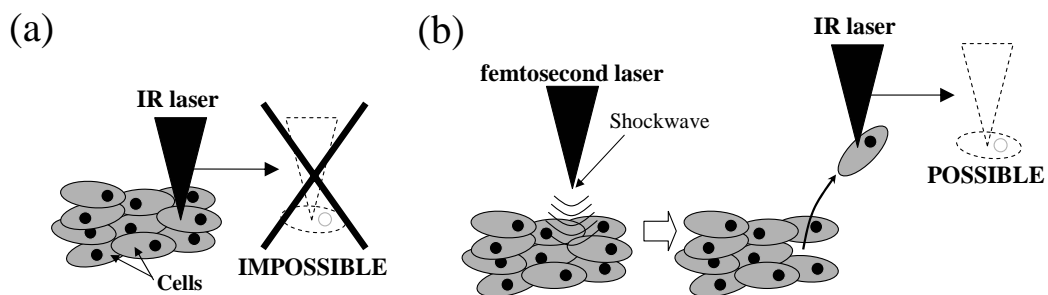


Figure 1 Single cell manipulation in tissue by laser trapping (a) and by laser trapping and femtosecond laser induced shockwave (b).

METHODS AND MATERIALS

A regeneratively amplified femtosecond Ti: Sapphire laser (Spectra-Physics, Hurricane), whose center wavelength is 800 nm and pulse duration is 120 fs, was led to a microscope (Olympus, BX50F-3), and focused with an objective lens (magnification is 100x, numerical aperture is 1.25.). The laser focal point is adjusted to the image formation surface of the microscope by tuning a collimator lens. The laser power under the microscope is tuned to an order of 0.1 $\mu\text{J}/\text{pulse}$ by a half-wave plate and a pair of polarizer. Liquid culture medium containing polystyrene particle (diameter is 1 μm), fission yeast cell (*Schizosaccharomyces pombe* (h⁻)), or pollen of buckwheat was sealed in a 100 μm space between a slide glass and a cover glass and set on the microscope stage. The real-time behavior before and after the laser irradiation was monitored using a color CCD camera (Flavel, HCC-600).

RESULTS AND DISCUSSION

1. Transfer of micro polymer particle by femtosecond laser induced shockwave

The micro polystyrene particle was dispersed in a culture medium and single shot of the femtosecond laser was irradiated with 1 sec. interval. The motions of the particles are shown in Fig. 2 (a) and the time evolution of the distance between the laser focal point and the particle was plotted in Fig. 2 (b). An obvious position shift of the particle (L) was observed immediately after the irradiation. The shift of the particle was decreased with increasing distance (R_0) between the initial position and the focal point. It means that particles floating in the culture medium were pushed by the shockwave. The force of the shockwave is proportional to the amount of the particle, so that the relation between L and R_0 will be due to a three dimensional diffusion of the shockwave force. The effective area of the shockwave was within 30 μm from the laser irradiation point under the present condition. In comparison with force of a conventional laser trapping, the force of the shockwave is enough larger than that of the laser trapping, because the initial velocity of the particle, which is estimated to be over 3 mm/sec at R_0 of 6 μm , is extremely larger than the velocity when the particle is transported in water by the laser trapping (6).

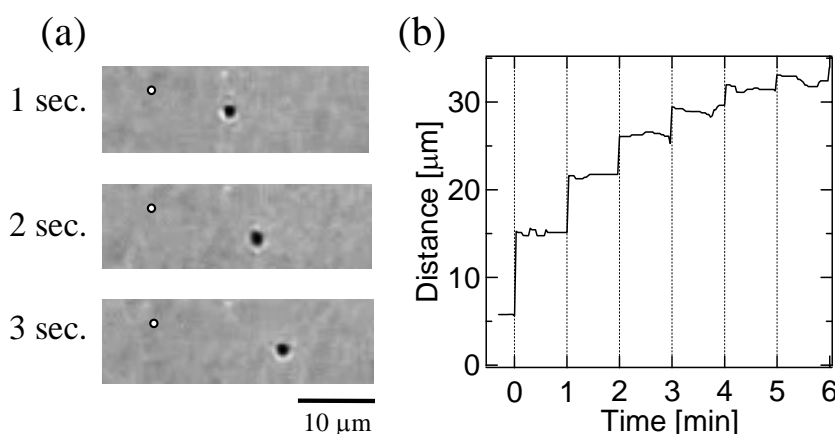


Figure 2 (a) Microphotographs of polystyrene particle into a culture medium after femtosecond laser irradiation. Femtosecond laser was irradiated with 1 sec interval. The white circles indicate focal point of the femtosecond laser. The particles look like black circle. (b) Time evolution of the distance (R) between the particle and the focal point. Pulse energy of the laser is 0.18 $\mu\text{J}/\text{pulse}$.

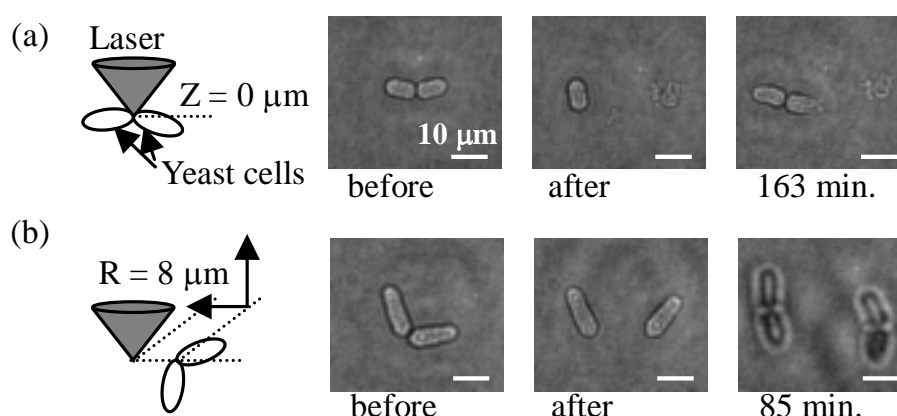


Figure 3 Illustration and microphotograph of femtosecond laser irradiation to fission yeast cells and growing process after laser irradiation. (a) The laser was focused at the interface between cells directly. (b) The laser was focused at 8 μm above the interface. (See text.) Pulse energy of the laser is 0.06 $\mu\text{J}/\text{pulse}$.

2. Nondestructive separation of living fission yeast cell

The cell division of the fission yeast cell was conducted symmetrically and two daughter cells of the same size were produced. When the laser was focused directly on the interface between the pair of cells as shown in Fig. 3 (a), the pair was separated immediately after laser irradiation and the right cell lost their silhouette. In the growing process after the laser irradiation, significant change of the right cell was not observed, although, for left cell, a self multiplication was observed at 163 min after the irradiation. It means that the right cell was filled by the damage of the laser. On the other hand, when the distance between the laser focal point and the cells was adjusted to be 8 μm in the focal plane (Fig. 3 (b)), although the similar cell separation was observed immediately after laser irradiation, self multiplications of both cells were observed at 120 min. Under this condition, as the laser pulse dose not directly irradiate to the cells, the separation will be initiated by the laser induced shockwave. Therefore, nondestructive separated living cell is achieved by the laser-induced shockwave. Previously, we have also tried to separate the pair of present yeast cells by using dual beam laser trapping system (15), however, it was not impossible. This result obviously indicates that the manipulation using the shockwave is effective technique to manipulate single cells.

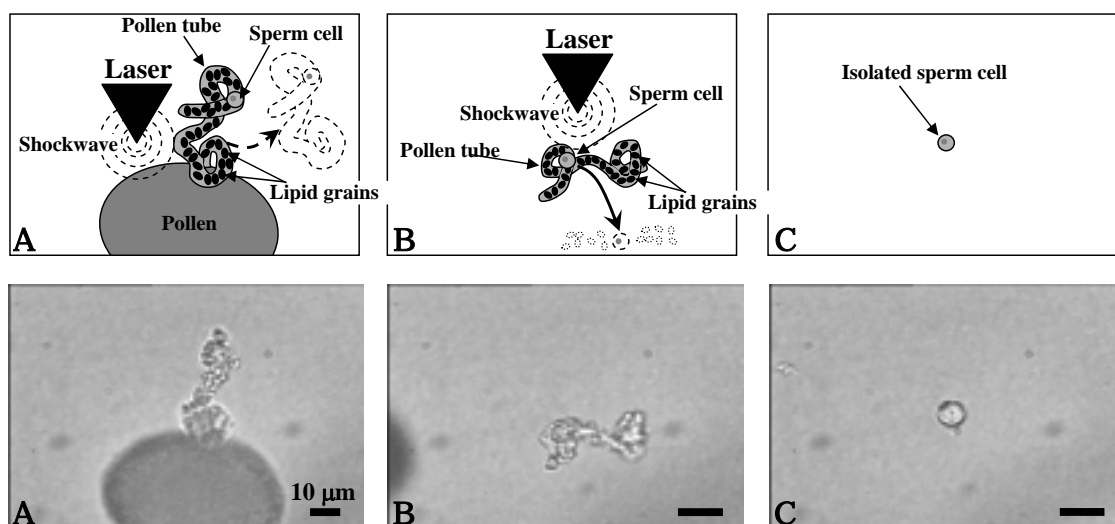


Figure 4 Illustration and microphotograph of separation of sperm cell in pollen tube. Pulse energy of the laser is 0.39 $\mu\text{J}/\text{pulse}$.

3. Nondestructive isolation of sperm cell in buckwheat

The manipulation using the shockwave was applied to isolation of sperm cell from pollen tube of buckwheat. The procedure of the isolation is shown in Fig. 4. The pollen tube is stuffed with much amount of lipid grain and one sperm cell which is observed at the head of the tube. Firstly, the shockwave was affected to the interface between the pollen and pollen tube, and the tube was separated from the pollen (Fig. 4 A). Secondly, the shockwave was affected to the pollen tube. As the membrane of the tube is brittle, lipid grains and the sperm cell were scattered from the tube (Fig.4 B). Finally, the sperm cell was divided form lipid grain by using the laser trapping (Fig. 4 C). This procedure was completed within 5 minute. The viability of the isolated cell was confirmed by fluorescein diacetate protocol. In conclusion, we succeeded in isolation of the sperm cell from the pollen tube by combining the shockwave manipulation with the laser trapping manipulation.

Normally, isolation of the sperm cell is preformed by a procedure using enzyme to break the membrane of the pollen tube, however, this procedure is very difficult because the enzymes also damage the membrane of the sperm cell. Hence, the present procedure is considered to have a new potential in the isolation. Further investigation for biological application of the shockwave has been conducted for not only plant cell but also animal cell.

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