Development of novel drug delivery system by using a pulsed laser with microparticles

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The introduction of exogenous materials into the cytoplasm of living cells is often useful in medicine, cellular biology and molecular biology. A number of methods involving chemical, viral or physical approaches has been developed to transfer DNA and other macromolecules into mammalian cells, although each has limitations.

Some investigators have reported laser-based systems for obtaining membrane permeability. Kurata *et al.* demonstrated that foreign DNA can be introduced into a target cell using a laser microbeam. According to them, when the laser beam was precisely focused on the cell membrane, the membrane at the site of the beam impact was modified through a local thermal effect. Since such "microbeam pricking" can be performed through the walls of culture dishes, it reduces the possibility of contamination accompanying many other *in vitro* methods. However, this technique has several shortcomings, including the impossibility of injecting molecules into non-adherent cells; the number of cells that can be injected is also a limiting factor.

The aim of this study is to develop a new method for permeabilizing cell membranes using visible laser light that is not focused on the cell surface. It was expected that the goal of membrane permeabilization could be achieved by the addition of prestained particles combined with exposure of the cell suspension to visible light. Namely, when light enters medium containing both dyed particles and cells, the light is absorbed by the particles, producing local photothermal and photomechanical stresses (*e.g.* temperature increases, cavitation, and pressure waves). The localized stresses alter the plasma membrane permeability of the cells without affecting their viability. Because these stresses dissipate within a few tens of nanoseconds, the damage is only very local. And, since this method does not require focusing light on the cell surface, it is possible to treat many cells at once.

Latex particles have been used in numerous applications in the biomedical and biotechnology fields, such as for phagocytosis assaying, immunoagglutination tests, biological cell labeling, and drug-delivery systems. The polystyrene latex adopted in our investigation sticks non-specifically to many surfaces and molecules because of its hydrophobic surface.

In this report, we describe a laser-latex combination system that enables membrane-impermeable molecules to penetrate cell membranes. Laser light (Q-switched Nd:YAG laser, 532.5 nm) was used to irradiate a mixture of commercial latex particles (blue dyed, 1 μm in diameter) and mouse fibrosarcoma (Meth-A) cells. After irradiation, membrane permeability was evaluated by flow cytometric assaying using propidium iodide (PI) and fluorescein diacetate (FDA). The proportion of permeabilized-resealed cells was affected by changes in the light

intensity (~ 780 mW/cm^2), the irradiation time (~ 240 s), and/or the particle concentration (~10^9 particles/ml). The permeability persisted up to 20 min after light irradiation. Near the sites of individual particles, the permeability of the cell membrane is modified, probably due to localized temperature changes. These results suggest that this laser-induced permeabilization strategy constitutes a new means of delivering exogenous materials into living cells.