Femtosecond laser photoporation of nanoparticles into vital cells

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Statement of Purpose: The transfer of membrane impermeable substances such as genes, indicators, antibodies and macromolecules into vital cells is a ubiquitous problem in cell biology. Target specific cells for drug delivery is even more challenging, since most of the current technologies are based on treating global populations of cells. Photoporation using lasers offers unique features such as cell specificity with precise controlled conditions and high spatial resolution. Minimally invasive femtosecond lasers allow nanoscale transfer of macromolecules without compromising the cellular viability (1). In this study, we used a femtosecond laser to transiently perforate the cellular membrane and investigate the uptake of 200nm FITC conjugated polystyrene beads.

Methods: The photoporation set-up consisted of an inverted microscope (Olympus IX81) coupled with femtosecond Ti:Sapphire laser (Coherent Inc, 800nm wavelength, 100fs)) . Mouse NIH3T3 fibroblasts were cultured on a glass coated with poly-lysine (MatTech Corp.) in Dulbecco's modified Eagle's medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum (Gibco) under 5% CO_2 atmosphere at 37°C. The cell culture dish containing fibroblasts was brought in contact with an oil immersion lens ("Fluar", 100x/1.30, Carl Zeiss Inc.). The laser spot was focused on the cell membrane from under the bottom of the glass petri-dish and the location of the laser spot was marked. A polystyrene nanoparticle containing a fluorescent dye (Molecular Probes, F8811, diameter: 200 nm, excitation/ emission: 505/515nm) was dispersed in the culture medium (50µl of 1:2000 bead solution). Target cells were positioned at the laser beam focus by manipulating the microscope stage on which the petri-dish was placed. (spatial resolution in XY-50nm; Z-10nm). The plasma membrane of the selected cell was exposed with optimum laser conditions (power:-30mW; exposure time:-10 ms; focal spot size: ~400nm) to generate a small pore, which allows small volumes of media containing the polystyrene nanoparticles to enter the targeted cell. The cell morphological changes induced by the femtosecond laser optoporation were monitored by a real-time CCD camera for 10min.

Results: Transmission image of a 3T3 fibroblast on glass petri-dish before the photoporation experiment is shown in Fig. 1A. We observed Brownian motion of 200nm nanoparticles throughout the media when we added the nanoparticle solution to the petri-dish. Before the laser exposure, there was not a single bead inside the cell; however several nanoparticles could be seen floating around the adhered fibroblasts (Fig.1B) (White spots surrounding the cell are due to florescence of the nanoparticles whose peak is at 515nm). Upon exposure to optimum laser conditions (Methods section) on a specific spot on the cell membrane, the nanoparticles in the media almost immediately go into the cell. Once inside the cell, the Brownian motion stops completely or is drastically reduced as compared to nanoparticles outside the cell. Cells were viable after performing photoporation experiment on the same cell at 3 different locations (Fig. 1C) and the pores seem to close following the closure of the shutter, suggesting both precise and transient nature of pore-creation. At optimum laser parameters, the fluorescence of nanoparticles was observed clearly inside cell in the XY plane (Fig. 1B). It is worth noting that the injection was only observed near the laser focal point and nanoparticles can also be traced at various Z sections inside the cell. Without laser exposure, the cells appear healthy for more than 2 hrs. There was no sign of endocytosis of nanoparticles or cell death during the present experimental time span (1hr). With lower than optimum laser exposure, the nanoparticles did not enter the cell; however nanoparticles were found to be drawn towards the laser focus spot. For higher than optimum laser exposure, the cellular viability was clearly compromised demonstrated by cell blebbing, an indication of cell stress and onset of apoptosis. White arrow in Fig. 1D shows a hole created by higher laser intensity leading to cell curling indicating apoptosis.

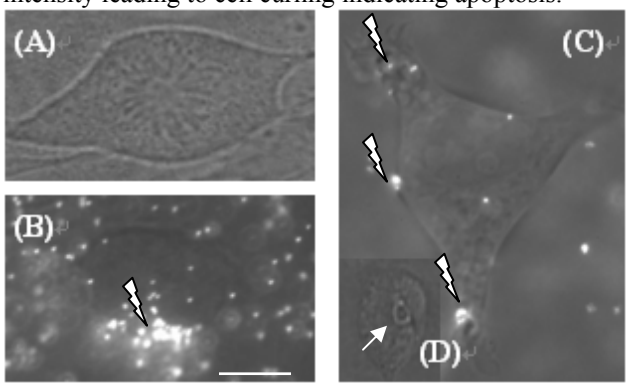

Figure1: Photoporation of mouse fibroblasts using femtosecond laser. (Scale:10µm) Lightning symbol indicates the location of laser exposure. Arrow represents a hole created by excessive laser exposure in (D).

Conclusions: We demonstrated the transfer of 200nm polystyrene beads into vital 3T3 fibroblasts via a transient opening of the cell membrane using femtosecond laser photoporation. Control experiments were carried out to unambiguously demonstrate that the interaction of the laser beam with the cell membrane is responsible for cell photoporation. We are currently optimizing various parameters such as laser exposure time, laser power, pore size (dyes, 20nm beads), transfer rate etc. Nanoparticles can be surface modified with DNA for gene transfection applications on various types of cells. This technique can be useful to deliver slowly diffusing macromolecules across the cell membrane. This could be a useful tool to load surface-functionalized beads with diverse materials in a contact-less, minimal invasive fashion.

Reference: Konig K. Journal of Photochemistry and Photobiology B: Biology 81 (2005) 136-142