Laser-based transfection with conjugated gold nanoparticles

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Received July 3, 2009

The irradiation of cells combined with the immunoconjugate of gold nanoparticles by the short pulse laser can make the plasma membrane be transiently permeabilized, which can be used to transfer exogenous molecules into the cells. We explore this technique as a novel gene transfection method for floating cells. Three different floating cells exposed to the laser are selectively transfected with fluorescein isothiocyanate-dextran, antibody, and green fluorescent protein (GFP) coding plasmids, and the viability of cells are determined by propidium iodide. For fluorescein isothiocyanate-dextran, the best transfection efficiency of 65% is obtained; for the antibody, it is 74%; whereas for the green fluorescent protein coding plasmids, a very small transfection efficiency is gained. If the transfection efficiency is improved, gold nanoparticles will be very useful as mediator for gene transfection in living cells.

OCIS codes: 170.2520, 140.3440, 350.5340.

doi: 10.3788/COL20090710.0898.

Intense efforts are being made in the interaction of laser energy with different absorbing nanoparticles in both nanotechnology and laser medicine, but most groups use this method to increase the sensitivity of photoacoustic diagnosis[1] or aid in therapy[2]. Recently, this technique has shown the capability to obtain plasma membrane permeablility[3]. In particular, we demonstrated that the laser-nanoparticle system could load cells with exogenous molecules[4]. The laser-assisted gene transfection is attractive as a new physical method for targeted gene therapy because of the high spatial controllability of laser energy[5], and most reports on laser-mediated gene transfer have dealt with single-cell-basis transfection by direct irradiation of the cell membrane with a focused laser beam with very low throughput[6,7]. Gene transfection is a basic technique in biological and medical research; but for some of the cell lines, such as cancer cell and floating cell lines, there is no safe and efficient transfection method, especially for *in vivo* applications. Although virus based transfection method has very high efficiency, and it is the most widely used transfection method at present, but it also has safety problem. So, there is a need to develop a safe, efficient, and high throughput method to deliver foreign DNA into cells or tissue. In this letter, we develop a new laser-based gene transfection method by which a large number of cells can be treated simultaneously. Firstly, we show that 10000-dalton (1 dalton = 1.65×10^{-24} g) small molecule fluorescein isothiocyanate-dextran (FITC-D) can be delivered into cultured cells by using the laser-nanoparticles system with high efficiency. Then for the large molecules such as antibody MIB1, we also get good results. But for plasmid coding enhanced green fluorescent protein, we get the same result with lower efficiency.

Three types of lasers were used in this work. two Q-switched frequency-doubled Nd:YAG lasers generating 6-ns pulses at 532 nm with different profiles were employed for irradiation. One laser had an unstable resonator with a focus beam profile of about 3 mm in diameter (Surelite I Continuum), and single pulses were used to irradiate the samples. The second laser had a TEM_{00} Gaussian beam with a diameter of 1.2 mm, which scan over the sample with a pulse frequency of 20 Hz. The third laser was a continuous-wave (CW) pumped mode-locked Nd:YLF laser producing 35-ps pulses at 527 nm with scanning irradiation manner. On the other hand, three types of floating cell lines including Hodgkin's disease cell line L428, the human large-cell anaplastic lymphoma cell line Karpas 299, and myeloid cell line KG1 that were all obtained from Forschungszentrum Borste, Germany, were selected. All these three cells were routinely grown in suspension culture in a RPMI 1640 (1×) with HEPES buffer and L-glutamine medium supplemented with 10% fetal calf serum, antibiotic/antimycotic solution (100×) in a 37 °C humidified incubator (5% CO_2, 95% air). For all experiments, the configuration was similar to the setup used in Ref. [8].

The gold nanoparticles with the average sizes of 30 and 15 nm were chosen covered with different antibodies for targeting cells. For Karpas 299, L428, and KG1 cell lines, the antibodies were ACT1, BerH2, and TüK1, respectively. Through these antibodies, the gold nanoparticles could be bound to cells. For the experiments, cells at the logarithmic growth phase were spun down at 1400 rotations per minute (rpm) and 20 °C for 5 min, and then resuspended in phosphate buffered saline (PBS) with certain concentration. The cells were then incubated with different immuno-gold particles according to cells in incubator for 20 min. For the small molecules, just before the irradiation, 10000-dalton FITC-D was added as a probe for membrane permeabilization. About 30 min after irradiation, the cells were resuspended in PBS containing propidium iodide (PI) to assay the cell death. Whereas for the plasmid DNA, just before or after the irradiation the plasmid DNA was added to the cell solutions. And 24 h after irradiation, the expression of enhanced green fluorescent protein (EGFP) in the cells was observed by use of a fluorescence microscopy

(OLYMPUS BH2-RFL-T2), and the fluorescence images were recorded using a charge-coupled device (CCD) camera.

During our experiments, the samples were irradiated in wells with a diameter of 2 mm, which were custom-made in a 25 × 75 (mm) slide of optical glass (Hellma). Each of the 18 wells took a sample volume of 4.0 μL. For the small molecules, the transfection efficiency and the viability of cells were assessed with a flow cytometry (FACSCAN, Beckman Coulter). Fluorescence signals were stored and processed with software program. A total of 5000 cells were examined per sample.

In the experiments we have used two kinds of gold nanoparticles, three laser sources to irradiate three types of cells, respectively. And we got the best transfection result by the nanosecond laser with scanning method, in which the cells were conjugated with 30-nm gold nanoparticles. Figure 1 shows the transfection efficiency and cell viability of the three cell lines with the nanosecond laser by scanning method.

Since we got the excellent results with the smaller molecules, the larger molecules such as MIB1 antibody (molecular weight 1.5×10^5 dalton) were used as exogenous material to be delivered to cells. In the experiments, 15- or 30-nm BerH2-Au was bond to L428/Karpas299 cell line, and different energy powers were used to irradiate the cells. MIB1 antibody is a kind of nucleolus antibody. When the antibody was transferred to cells, it can mark the nucleolus. For measuring the membrane permiabilization by flow cytometry, MIB1 was marked with Alexa 488 before it was delivered to the cells[9]. In this group of experiments, we got the best transfection ratio of 74%. And we have investigated the tranfection results using a multiphoton fluorescence microscope system, the results are shown in Fig. 2. In pseudo-color images, we can see that the cells are coated by gold nanoparticles (golden yellow), green denotes the cell tranfected by FITC-D, and blue denotes the cell tranfected by MIB1-Alexa 488.

We further try to transfect EGFP coding plasmid in these floating cells. We also used three laser sources to irradiate the three kinds of cells. During these experiments, for all these three cells the experiment condition is the same that for the small molecule transfection, but we add the plasmid DNA either just before or after irradiation in order to compare the different results. Also, 24 h after laser irradiation, the expression

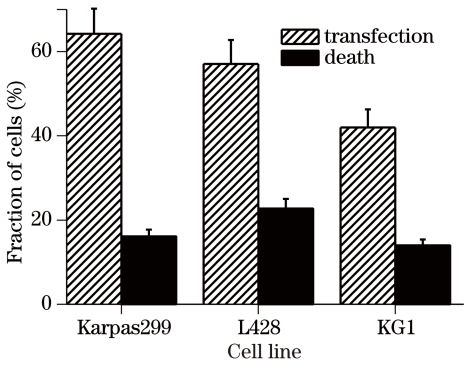

Fig. 1. Transfection efficiencies and cell death rates of different cells with nanosecond pulse laser by scanning irradiation way.

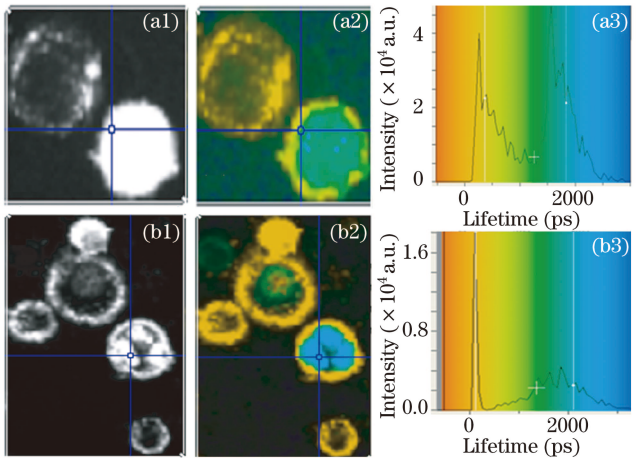

Fig. 2. Multiphoton fluorescence imaging of the Karpas 299 cell tranfected by different molecules. (a) Au30-ACT1 coated cell transfected by FITC-D after laser irradiation; (b) Au30-BerH2 coated cells transfected by MIB1-Alexa 488 after laser irradiation. (a1) and (b1) are intensity images, (a2) and (b2) are pseudo-color images, (a3) and (b3) are corresponding fluorescent life histograms. Exciting light source: 50 nm, 20 mW; collection: 7.4 s/frame, 1 min. (a1), (a2): 600×; (b1), (b2): 400×.

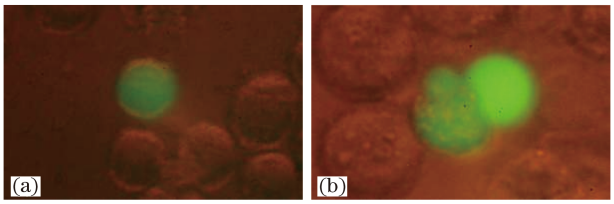

Fig. 3. Fluorescence images of the transfected cells (Karpas 299) with the plasmid coding for EGFP. (a) With Fugene transfection method and (b) with laser-particles method.

of EGFP in the cells was observed by fluorescence microscopy, and the fluorescence images were recorded by a CCD camera. For the picosecond laser, we have not found the expression of EGFP in three cell lines. Furthermore, for the KG1 cells, we also have not observed the transfected cell with three laser sources. However, we observed transfected cells in the other two cell lines irradiated with the other two nanosecond laser sources. Figure 3 shows the photo of the transfected cells. For comparing the transfection efficiency with traditional method, we also take the photo of the transfected cells with Fugene transfection method.

Figure 2 give the experiment results of Karpas 299 cell line, and the L428 cell line has similar results. Under the condition that just before irradiation the plasmid is added, we can get better result for both cells. And for transfection, the scanning way is better than single laser pulses.

Up to now, we have demonstrated the *in vitro* transfection of EGFP coding plasmid in Karpas 299 and L428 cell lines by using immuno-nanoparticle and laser light. We got 1% gene transfection efficiency in most cases. However, to the best of our knowledge, the laser method using the irradiation of selective cell targeting with light-absorbing particles for gene transfection has not been reported yet. Furthermore, since there was no good method for transfecting floating cell at present, the method described here would give a new idea. The mechanism

of laser-particle-induced gene permeation through the plasma membrane is not clear, but there are three possibilities: denaturation of membrane proteins because the receptors by which the gold particles are bond to the cells are membrane proteins, transient disruption of the plasma membrane by plasma formation, and membrane disruption by cavitation[10]. Zharov *et al.* have shown the microbubble formation around cellular absorbing zones with photothermal technique[11]. In addition, the mechanism of laser-particle-induced gene transfection is not similar to that of laser-induced stress waves (LISWs), because the membrane recovery time is so different[11]. In our experiments, we have used three kinds of cells and got different transfection efficiencies with different irradiation conditions. It is possible that the different distributions of the receptors on the cell surface and different structures of the receptors influence the transfection efficiency[4].

In any cases, a high local temperature is created in and around the particles during membrane permeabilization[12,13]. Estimates of the temperatures[14] for our experimental conditions give a temperature increase by 900 K when 15-nm particles are irradiated with a 10-mJ laser, while the 30-nm particles are heated by 3600 K. These temperatures are high enough to evaporate water in a layer around the particles, which creates a rapid expanding bubble. Such bubbles with submicrometer diameter around gold nanoparticles were experimentally observed under laser irradiation[11]. Additionally, the gold melts and breaks up into smaller particles[15]. Particle fragmentation was observed at a radiant exposure starting from 80 mJ/cm^2 for 40-nm particles[16]. Since under nanosecond irradiation the particle temperature scales with the square of the particle diameter, for particles below a certain diameter, the melting temperature will not be reached. Therefore, after a certain number of pulses, no further effect is expected when all particles are fragmented. Cavitation bubbles were also proposed by Pitsillides *et al.* as the mechanism for cellular effects caused by laser-irradiated nanoparticles[3]. Although bubble formation does certainly occur under our irradiation conditions and spatial extension of the bubbles[17] is expected to be larger than the volume which is heated by the particle, we cannot rule out that direct damage to the targeted protein contributes to the membrane permeabilization or cell killing.

In conclusion, we give the description of the combination of immuno-particle and laser beam for delivering the EGFP coding plasmid into floating cells. In our technique, the effects are localized to particle contact sites, thereby limiting the size or extent of the plasma membrane damage sites. Compared with LISW transfection method which is only suitable for attached cells, our method can transfect not only attached cells but also floating cells. Although the transfection efficiency of floating cell lines is very low at present, this new idea could be developed in the future for much higher efficiency. For example, the efficiency can be improved by using more effective antibodies, choosing more suitable nanoparticle size, and so on. Furthermore, because of the invasion depth and non-damage to normal tissue of low energy laser, this method would be a promising method for *in vivo* transfection for gene therapy in the future.

The authors thank Gereon Huettmann, Ramtin Rahmanzadeh, and Astrid Rodewald for their help during experiments. This work was supported by the China Scholarship Council, the National Natural Science Foundation of China (Nos. 60578026 and 60878056), and the Natural Science Basic Research Project in Shaanxi Province, China (No. 2009JQ4013.)

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