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SHORT COMMUNICATION

A PROPER MIXTURE OF FOOD OIL AND LECITHIN IS AN EXCELLENT MEDIATOR FOR GENE-TRANSFER TO MAMMALIAN CELLS

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ABSTRACT

In order to facilitate genetic studies in mammalian cells, we developed a cost efficient lipid-mediated plasmid DNA transfection protocol utilizing food oil as a transfer-mediator. The intention was to create and/or improve a cheap and efficient method. Compared to the conventional transfection using commercially available lipofection-specimen, this procedure remarkably enhanced the delivery of a GFP reporter plasmid into HEK293 cells. Our technique of gene delivery is adaptable for large scale production since the costs of the reagent is two to three orders of magnitude cheaper than conventional commercial available reagents.

A number of transfection techniques are available to introduce foreign DNA into cells (Roy *et al*., 2005, Halterman *et al*., 2009, Cao *et al*., 2010). Among them, the advantages of using nonviral delivery compared to viral vector include decreased immunogenicity and less mutagenesis due to the genome insertion, however, the disadvantages of nonviral delivery may include decreased transfection efficiency. High efficiency, low toxicity and cost efficiency are still desirable properties for improved nonviral transfection techniques. Although various studies have examined the gene-transfer efficiency of nonviral delivery to cells, few have focused on a direct comparison including cost-performance efficiency of the techniques. It is now difficult to continue molecular biological study, in which gene transfer technique is almost necessary, without using expensive transfection reagents. This situation had encouraged us to discover novel and cutting-edge techniques as well as optimize the protocols, which increased those efficiencies.

In order to evaluate different transfection methods regarding their potential for transient protein expression, we compared the reagents made of 38 kinds of commercially available food oils (S1-S38) those were purchased from food market in Japan. The newly designed transfection-reagent, which was called FOL solution as it was made of food oil and lecithin, were produced as bellow.

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FOL solution preparation recipe:

1. In a sterile microfuge tube, mix 75 mg of lecithin with 500 µl of food oil (S1-S38), 500 µl of ethanol, 50 µl of 1mM sodium cholate and 20µl of alpha-tochopherol.
2. In the other sterile microfuge tube, mix 35µl of above the solution with 500 µl of ethanol, 1µl of glycerol and 3 µl of PBS.

To optimize transfection efficiency we varied the DNA (0.1-2 µg) and the reagent (0.5-5.0 µl) concentrations in transient transfection experiments. The condition of preincubation was also optimized. Consequently the transfection protocol should be as below:

1. Dissolve 500 ng of plasmid DNA in 100 µl of serum free cell culture medium (DMEM or RPMI).
2. Add 1 µl of FOL solution, and mix well. Incubate it at 45°C for 20 min.
3. The above DNA-FOL mixture (~101 µl) is slowly added over the 35 mm monolayer of pre-washed cells to be transfected.
4. Incubate the mixture for 60-90 min at 37°C in a CO2 incubator.
5. Add the culture medium containing appropriate concentrations of serum.
6. 2-3 days after transfection, the cells could be examined for protein expression.

As shown in Figure 1, the transfection efficiency of this system has been evaluated by examining the expressional protein level via western blots. Figure 1 and our preliminary experiments indicated that the FOL transfection reagent with S3 food oil (S3-FOL) seemed the most excellent gene-transduction efficiency into

** Hitomi Yoshida and Satoru Matsuda contributed equally to this work.*

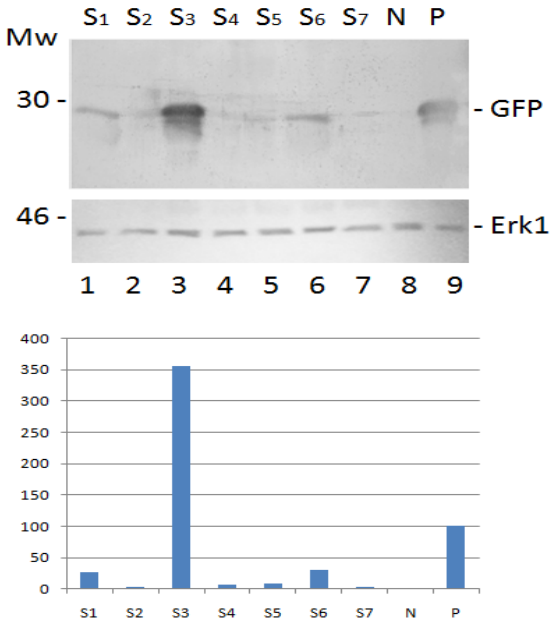

Fig.1. Comparison of transient transfection efficiency with newly prepared reagents containing various food oils (S1-S7), naked DNA plasmid (N) and a standard commercially available lipofection reagent (P). HEK 293 cells were transfected with GFP expression plasmid using the optimized protocols for the transfection. Protein expression level was evaluated by western blot with anti-GFP antibody (SantaCruz) 48 hr after transfection.
Western blot with anti-Erk1antibody was also shown in the middle panel, as equal levels of protein loading. Molecular markers (Mw) are indicated in the left in Kd. % amount of GFP expression (arbitrary units) are shown on the bottom chart.

HEK293 cells. As shown in Figure 2, fluorescent and phase contrast microscopy also showed the gene delivery-virtue of the S3-FOL reagent. Viability of cell population was acceptable with values above 96% (data not shown). In addition, the expression of the GFP was determined by FACS to investigate the excellence of the transfection efficiency (Figure 3). Several commercially available transfection reagents have also been compared to our S3-FOL, however, the FOL was the best on all of our transfection efficiency evaluations. We were able to increase transient expression of GFP reporter plasmid gene by an average of hundred-fold compared to naked DNA transfection (data not shown). We observed neither a decrease in transfection efficiency when cell grew confluent nor inhibition of transfection by serum. The transfection efficiency was not improved upon higher amount reagents. Likewise, an increase of plasmid DNA did not improve the transfection efficiency (data not shown).

The cytotoxicity of our reagent was checked by counting viable cells after the transfection using trypan-blue as vital stain. We monitored the number of viable cells by trypan blue staining after 24 hr incubation with various concentrations of reagents. Three independent experiments were performed to determine the cytotoxicity. In preliminary those toxicity studies, all the reagents proved to be few toxic (the viability of all transfections was above 95%, data not shown), and no adverse detrimental effects had been observed in our in vitro experiments so far. This method may be suitable for

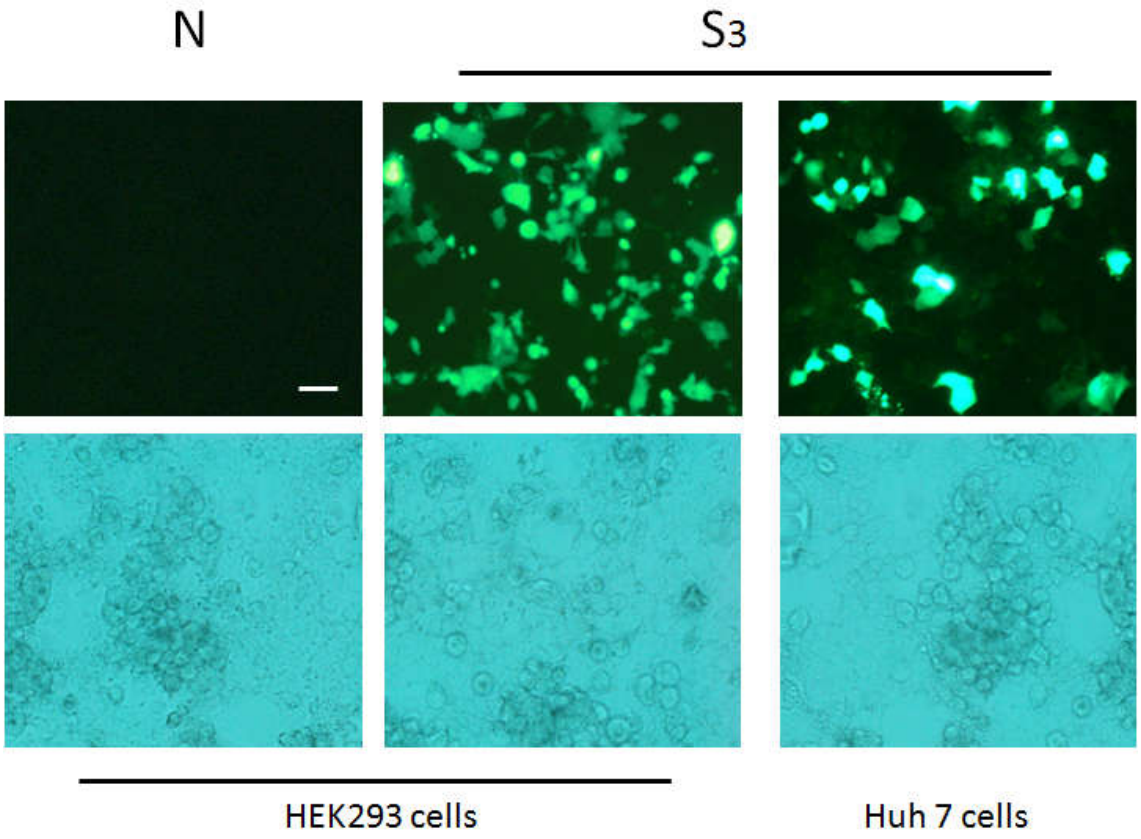

Fig.2. Expression of GFP in HEK 293 (left and middle panels) and Huh 7 hepatoma cells (right panels) by S3-FOL reagent as a delivery mediator. Fluorescence (upper panels) and phase contrast (lower panels) images are shown. A white bar in left-upper panel represents 20 micro meter.

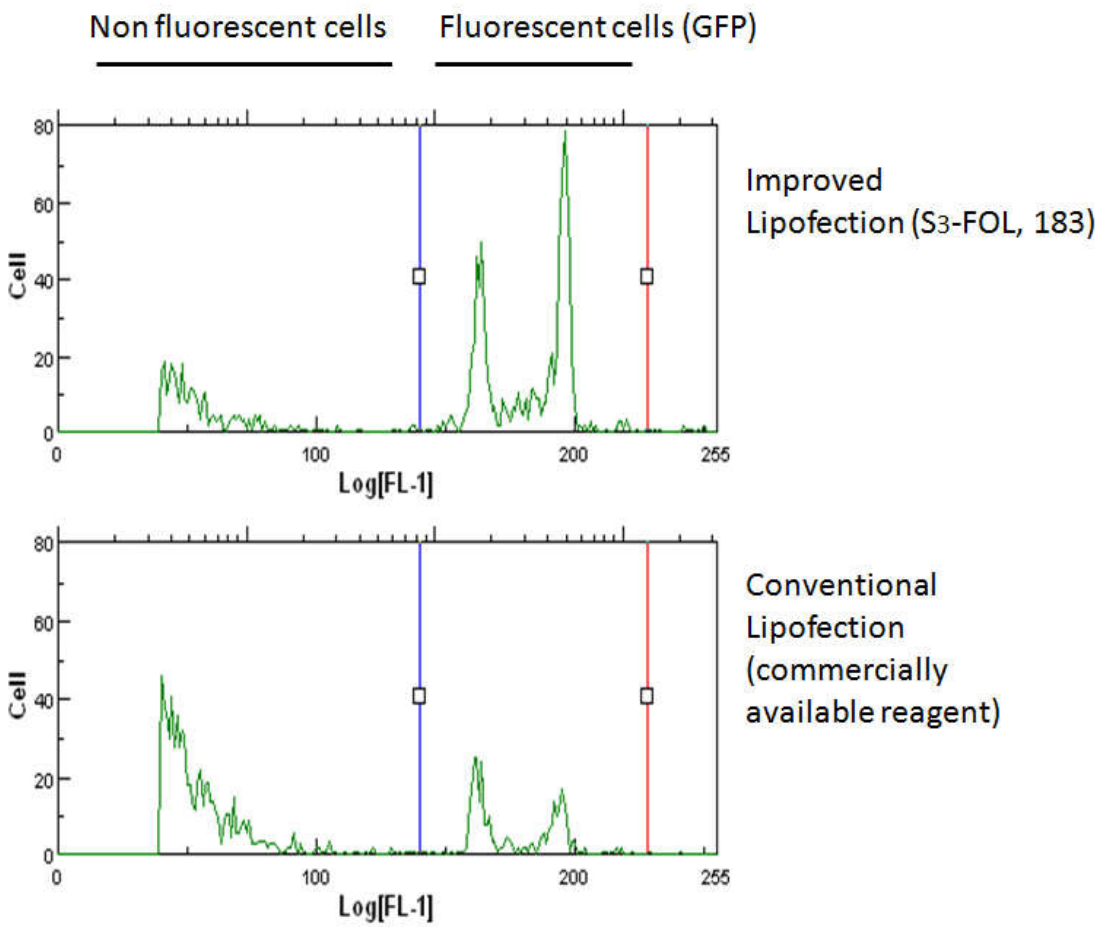

Fig.3.Measurement of GFP expression using flow cytometry analysis of HEK 293 cells transfected GFP expression plasmid with S3-FOL reagent (upper panel) compared to the conventional protocol (lower panel). The conventional lipofection was performed according to the manufacturer's instruction.

generation of stable cell lines. However, the formation of large aggregates that is inherent to this technique might cause a potential danger in case of in vivo experiments.

Generally the cationic lipids act destabilizing on the lipid bilayer and enable the DNA to escape from the compartment of lysosomal DNA-degradation (Wattiaux *et al*., 1997), however, the exact mechanism of the endosomal membrane crossing (Zuhorn *et al.*, 2005) and the DNA transfer across the nuclear membrane remain unsolved. Furthermore, it is not possible to predict the DNA delivery from the lipid character, since a variety of additional parameters such as cell type, culture condition, plasmid size and DNA property are probably of equal importance. The high efficiency of this method presented here may be due to the intrinsic membrane rupturing capability of cationic ingredient as a result of destabilizing the plasma membrane. The sequence of molecular events underlying the complex phenomenon of gene-transfer remains elucidative. However, it is commonly accepted that endocytosis is the main mechanism of the entry of DNA-reagent particle (Khalil *et al.*, 2006, Wittrup *et al.*, 2009). The system presented here should be feasible for applications that do not require targeting to a defined cellular property, because the uptake of the reagent seems to be independent of cell type.

The use of genes as drugs is still limited by the lack of low cost, efficient and nonhazardous vehicles. In this study, we developed a cost efficient lipid-mediated plasmid DNA transfection system based on nonviral lipofection technique utilizing food oil as a transfer-mediator that may offer an alternative to present systems. Compared to the conventional transfections using commercially available specimen (lipofection), the procedure remarkably enhanced the delivery of a GFP reporter plasmid. Our technique of gene delivery are adaptable for transient large scale production since the costs of the reagent is two to three orders of magnitude cheaper than conventional commercial available DNA vehicles. Furthermore, the toxicity is very low, probably because of its origin as a food ingredient.

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