Gene Transfer into Cells from Solid Surfaces and Its Application to In Vivo Systems

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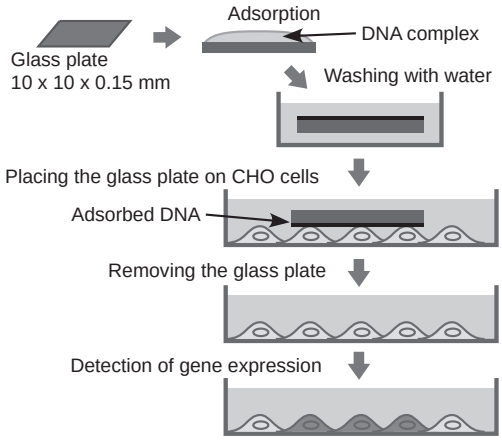
Plasmid DNA adsorbed onto a glass surface could be transferred into cells by pressing the glass onto the cells, and the gene encoded in the DNA was expressed in the cells. This novel transfection technique will be applicable to the preparation of cell microarray systems and for gene delivery mediated by a solid medical device such as a stent.

Highly efficient and functional gene transfection is a crucial technique, not only for molecular biology but also for gene therapy. In general, gene transfection is mediated by cationic liposomes and polymers, which form complexes with plasmid DNA and are then internalized into cells via the endocytosis pathway.[1] As a new in vitro transfection technique, gene transfection from a solid surface was reported by Sabatini et al.[2] They spotted plasmid DNA in a gelatin solution onto a glass slide. After treating the glass with a cationic lipid, cells were cultured on the slide. The plasmid DNA was internalized into cells that adhered within the spotted regions. This technique provides a high throughput transfection microarray to discover novel functions of genes. Another study on transfection microarrays for siRNA and nonadherent cells has also been reported.[3] On the other hand, in vivo gene transfection to vascular intima from a stent surface was reported.[4] Plasmid DNAs were immobilized on the surface of the stents with poly(lactic-*co*-glycolic acid) (PLGA), polyurethane, collagen, and gelatin and then transferred to cells of the vascular intima. Yamauchi et al. optimized DNA immobilization onto a gold surface for stent-assisted gene transfection by assembling, layer-by-layer, cationic lipids and the DNA, in in vitro conditions.[5] The layer-by-layer assembly of the DNA increased the amount of immobilized DNA on the gold surface. The DNA was transferred to the cells from the apical side by placing a DNA-immobilized gold wire onto the cell layer. In this study, to establish an easy and simple transfection procedure from a solid surface, we attempted to use naked glass and stainless steel as transfection devices for cultivated cells and tissue, respectively. This will allow us to prepare transfection microarrays in vitro and in vivo and to achieve gene delivery from a therapeutic solid device such as a stent.

Plasmid pCMV-luc DNA (1 μg/well of 24-well plate, encoding luciferase) alone and in complexes with protamine, jetPEI™ and Lipofectamine2000™ were adsorbed onto glass plates ($10 \times 10 \times 0.15\,mm^3$) for 30 min. After washing the plates with water, the glass plates were put onto CHO cells cultured in DMEM for 30 min then removed (Scheme 1). Cells from the plate adsorbing the DNA complex with Lipofectamine2000™ showed higher gene expression from the DNA than the DNA alone and the complex with protamine (Figure 1). Interestingly, the plate adsorbing the DNA alone showed significant gene expression, suggesting that free DNA



Scheme 1. Protocol for gene transfection mediated with a glass plate through the apical side of cells.

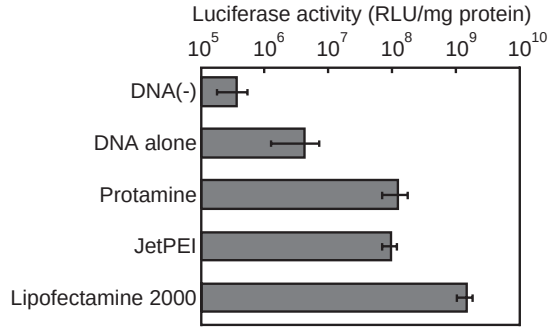


Figure 1. Transfection efficiencies mediated by pushing of the glass plates adsorbing DNA alone and DNA complexes with protamine and Lipofectamine2000™.

could bind to the surface of the glass plate and be successfully transferred into cells. As a result of reducing the amount of the DNA–protamine complex adsorbed onto the glass plates, 50 ng of DNA was sufficient to show significant gene expression (comparable to 1 μg of the DNA, Figure S1),[6] meaning that the amount of adsorbed DNA was about 50 ng even if we applied a larger amount of the DNA complex to the glass plates. Actually, it was too hard to determine the amount of DNA on the glass surface using an ethidium bromide staining method.

To confirm whether the DNA transfers into cells were mediated by direct transfer from the glass plate to cells or if the DNA was released from the glass plates before being internalized into cells as in a general transfection process, we applied the glass plate so that the adsorbed side was uppermost (opposite side from cells). As a result, Lipofectamine2000™ showed a high efficiency of transfection even when the adsorbed layer was upper-

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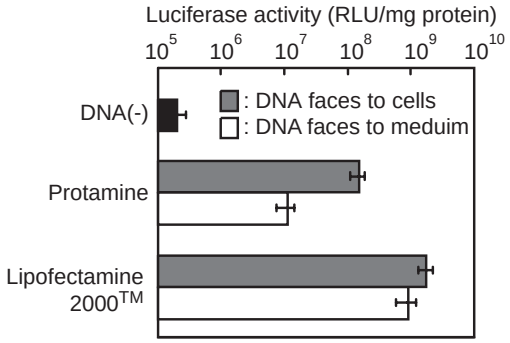

Figure 2. Effect of adsorbed side of the DNA on the transfection efficiencies. DNA complexes with protamine and Lipofectamine2000™ were adsorbed on the glass plates, and the plate was placed onto CHO cells so that the adsorbed layer faced either the cells or the medium side.

most (Figure 2). Thus the contribution to the overall rate of transfection of released DNA complexes from the glass plates is not be negligible. The use of Lipofectamine2000™ could be disadvantageous in preparing a transfection microarray with high spatial resolution even though the transfection efficiency was high. In the case of protamine, a 13-fold reduction in the amount of transfection was observed by turning the plate upside down. Thus, in the case of the protamine–DNA complexes, direct transfer of the DNA complex from the glass surface to the cells is probably the major contributor of transfection. In the case of free DNA, no gene expression was detected after turning the plate upside down (data not shown).

EGFP expressions mediated with the transfection system from the glass plates into CHO cells were observed under a fluorescence microscope. Considerable detachment of cells at the placing site was not observed, suggesting that cellular damage during the transfection process would be negligible. In all cases, positive cells expressing EGFP were observed (Figure S2). However, the percentage of expressing cells was less than 10%, even in the case of Lipofectamine2000™. The reason why high luciferase expression was observed is that a small number of cells expressed large amounts of the protein. To improve the transfection efficiency, an optimizing strategy to adsorb much more amount of the DNA will be necessary. Improvement to the flatness of the glass surface to deliver the DNA to the cells uniformly will also be required.

The transfection system from the apical side of cells has an advantage in the preparation of transfection microarrays on tissues because the DNA on the plates is placed directly onto the tissue compared with the conventional techniques achieved by seeding cells onto the arrayed surface.[2–4] Here, to demonstrate the construction of a transfection array on the tissue in vivo, DNA encoding β-galactosidase adsorbed onto a glass plate was placed and pushed onto the liver of a mouse under anesthesia. Unfortunately, staining of the treated liver with X-gal showed little gene expression at the site where the glass was placed on the liver (data not shown). To enhance the gene expression, electric pulses were employed to release the DNA complexes from the plate surface and permeate them through cell membrane. Since the glass plate is an insulator, a stainless steel plate ($10 \times 10 \times 2.0\,mm^3$) was used as a solid support instead of the glass plates. After casting 50 µL of 50 µg/mL DNA

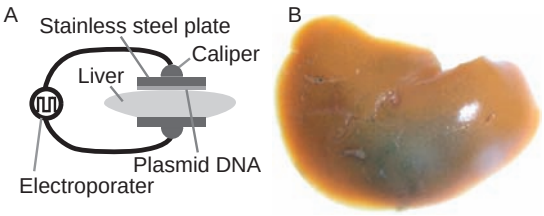

Figure 3. Gene transfection to surface cells of the liver achieved by pushing a stainless steel plate adsorbed with DNA encoding β-galactosidase and applying electric pulses (1 V/cm, 1 Hz, 5 ms, 8 pulses). Gene expression was detected by staining with X-gal of the whole liver.

solution onto the stainless steel plate, it was placed on left lobe of the liver. Electric pulses (1 V/cm, 1 Hz, 5 ms, 8 pulses) were applied to the left lobe with the plate by sandwiching with a caliper for in vivo electroporation (Figure 3A). After applying the electric pulses, significant gene expression (blue-stained cells) was observed at the pulsed area (Figure 3B). Stronger electric pulses e.g., 1 V/cm, 1 Hz, 10 ms, 8 pulses caused slight scorching of the liver and showed no gene transfection, and weaker electric pulses showed little gene transfection (data not shown). As a result of observation of a cross section of the liver, the blue-stained cells were observed within a region of 200 µm in depth (data not shown).

By spotting several genes on the plate, the transfection microarray will be constructed on tissues in vivo. This technique is also expected to be useful as a screening system for optimizing coating conditions for gene delivery from a stent surface. This easy and simple gene transfection system using glass and stainless steel plates, which works in vitro and in vivo, will be a powerful tool for screening novel functions of genes in cells. It could become a basic technique of gene therapy-mediated solid devices, such as gene eluting stents.

This research was supported by a Grant-in-Aid for Scientific Research (B) (No. 19300172) from the Japan Society for the Promotion of Science (JSPS).

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6 Supporting Information is also available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

Published on the web (Advance View) November 29, 2008; doi:10.1246/cl.2009.36