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# Magnetofection is an efficient tool for ectopic gene expression into oral cells

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**It is difficult to introduce DNA in non-invasive manner into oral cancer cells as well as primary cells for gene manipulation and expression *in vivo*. So far, several methods for a gene delivery have been performed to solve this problem. Magnetofection is one of the recent methods for gene transfer, and nanoparticles are applied under a magnetic field for DNA delivery. We investigated whether the magnetofection increases the efficiency of a gene delivery into several oral cell lines. By using a plasmid coding the green fluorescent protein (GFP), the efficiency of gene transfer by magnetofection was compared with those by using the calcium phosphate and the commercial transfection agent. Indeed, the magnetofection increased the green fluorescent signal in cells, suggested that this method apparently enhance the efficiency of gene delivery without any defects in various oral cancer cell lines. Finally, we have shown that magnetofection can be a useful technique for gene delivery to difficult-to-transfect cells to perform a functional study of genes *in vivo*.**

**Keywords: Gene transfer, Magnetofection, Oral cell**

## Introduction

Many of modifications of gene delivery techniques have been used to improve the efficiency of gene expression in mammalian cells (Haines et al., 2001; Gottschalk *et al*., 1996; Ross, 1993). The highly efficient transfection is important in approaches, where transfected gene overexpression is used as a tool for studying the functions of gene or protein as well as for therapeutic applications. The efficiency of gene transfer techniques is limited, because of insufficient enrichment of the applied gene vector at the target cells, its intracellular trafficking, and nuclear uptake. Therefore, although the successful gene delivery by non-viral vectors into various cell lines, so far only low efficiencies of target gene expression in cultured endothelial and epidermal cells have been reported (Edgell *et al*., 1998; Tanner *et al*., 1997; Teifel *et al*., 1997). So far, molecular approach by target gene manipulation was not much progressed in oral cells, because it has been difficult to introduce and to express genes. Recently, although the recombinant virus technique has been tried in several groups, the construction of the virus was tedious and inefficient (Leivonen *et al*., 2006).

Magnetofection has been developed for enhancing delivery of non-viral gene to target cells. Magnetic targeting of gene delivery is achieved by application of a magnetic field to super-paramagnetic iron oxide particles associated with the gene vectors. This method was reviewed in detail by Plank and colleagues (2003). The application of magnetofection to viral and non-viral vectors has been recently shown in cell culture experiments in human chronic myeloid leukaemia in blast K562 cells and in Swiss mice embryo NIH3T3 cells (Scherer *et al*., 2002).

In this study, we investigated whether magnetofection could be an appropriate tool to increase the efficiency of gene transfer for further molecular cellular research in oral cell lines, which are difficult for transfection. Based on this report, we are in the hope of studying function of genes, which involved in various oral cancer and disease.

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## Materials and methods

### Materials in this study

pEGFP vector and Calphos™ mammalian transfection kit were purchased from Clontech (Mountain View, CA, USA). Fugene6™ transfection reagent was purchased from Roche Diagnostics (Mannheim, Germany). MagnetoFACTOR™ plates and PolyMAG™ magnetic particles for magnetofection were from Chemicell (Berlin, Germany). Anti-GFP antibody (sc-8334) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). ECL reagents were obtained from Amersham Biosciences (Buckinghamshire, UK). For cell viability assay, 3-(4,5-dimethyl-thizaol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) was purchased from Sigma Chemical Co.

### Cell culture and gene delivery

Human cervical carcinoma (HeLa cells), human mouth epidermal carcinoma (KB cells), oral mucoepidermoid carcinoma (YD-15 cells), and squamous cell carcinoma (YD-38 cells) were maintained in Dulbecco's modified essential medium (DMEM, Hyclone) supplemented with 10% fetal bovine serum (FBS, Hyclone) and grown at 37°C in a humidified atmosphere of 8% $CO_2$. In 60-80% of confluency for transfection, cells were incubated with 4 μg DNA by using the calcium ion in HBS (Hepes-buffered saline) solution, Fugene6™ (the commercial transfectant), and PolyMAG™ (the magnetofection reagent), following the standard protocols of the manufacturer with modification. In brief, for HBS-calcium ion method, cells were cultured in DMEM contained 5% serum for 4 hours, and the DNA-calcium mixture was added. After 16 hours, cells were washed, incubated for 24 hours, and harvested for evaluation of gene expression (Jang *et al*., 2002). For transfection by using Fugene6™, cells were washed in serum-free DMEM and the DNA-transfectant reagent mixture was added. After 5 hours, DMEM contained 10% serum was added, and cells were incubated for 24 hours and collected for evaluation of gene expression. For magnetofection, DNA-PolyMAG™ mixture was added in culture and the culture dish was placed on the magnetoFACTOR™ plate for 10 min. Cells were incubated without plate for 24 hours and harvested for evaluation of gene expression.

### Fluorescence microscopy

Cells were assessed by fluorescence microscopy. Fluorescence intensity in transfectants was monitored by a Zeiss Axiovert 25 microscope equipped with a baseport mounted CellCam Twain CCD camera, and a fluorescence filter set for GFP signal was used for data acquisition. Image processing was carried out using ScionImage software for PC (Scion Corporation, Frederick, ML, USA).

### Western blot analysis

Cells were lysed in Nonidet P-40 extraction buffer (0.5% Nonidet P-40, 50 mM Hepes, pH 7.4, 150 mM NaCl, 1 mM DTT, 5 mM EGTA, 1 mM EDTA, 20 mM NaF, 20 mM β,-glycerophosphate, 0.5 mM $Na_3VO_4$, 20 mM p-nitrophenyl phosphate) supplemented with protease inhibitors. The cell lysate was centrifuged at 15,000 x g for 20 min, and the supernatant was recovered. Protein concentrations were determined using the Bradford reagent (Bio-Rad, Richmond, CA, USA). Total proteins (50 μg) were separated by 10% SDS-PAGE and transferred onto a PVDF membrane. Membranes were blocked with 5% skim milk for 1 hour at room temperature. Blots were incubated with primary antibody (1 : 1000 dilutions) for 2 hours at room temperature. Membranes were rinsed twice with TBST (5% Tween-20 in Tris-buffered saline, pH 7.4) and incubated with secondary antibody conjugated to HRP (1 : 1000) in a blocking solution for 1 hour at room temperature. The protein complex was visualized using the ECL reagent (Amersham Biosciences, Buckinghamshire, UK).

### Cell viability assay

The viable cell numbers were measured by a modified 3-(4,5-dimethyl-thizaol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. Briefly, cells (HeLa, KB, YD-15, and YD-38 cell) were seeded at an initial density of $1\sim2\times10^5$ cells/well in a 24-well plate. These cells were then transfected with PolyMAG™ transfection reagent according to the standard protocols of the manufacturer. After incubation for 30 hours, a final concentration of 5 mg/ml MTT was added to each well, and crystals absorbed in viable cells were finally dissolved in dimethylsulfoxide of 250 μl/well. Signals from the viable cells were determined at 540 nm.

## Results

Generally, for ectopic gene expression in mammalian cells, there were several non-invasive methods by using chemicals either hand-made or commercially available. However, these methods showed very low efficiency of gene transfer in most cell lines, especially primary cells. In this study we show the magnetofection technique for non-invasive gene transfer. As comparative studies, simple transfection by using calcium ion and chemical transfection reagents, CalPhos™ and FuGene6™, were performed. We used three kinds of oral cell lines: human mouth epidermal carcinoma (KB cells), oral mucoepidermoid carcinoma (YD-15 cells), and squamous cell carcinoma (YD-38 cells), and human cervical carcinoma (HeLa cells) as a control cell line, which is well-transfected by simple transfection with calcium ion (Jang, *et al*., 2002). The pEGFP vector encodes a red-shifted variant of wild-type GFP, which has been optimized for brighter fluorescence and higher expression in mammalian cells. Transfection efficiencies from those trans-fection methods were assessed at the same concentration of vector DNA (4 μg). After 24 hours of transfections, cells

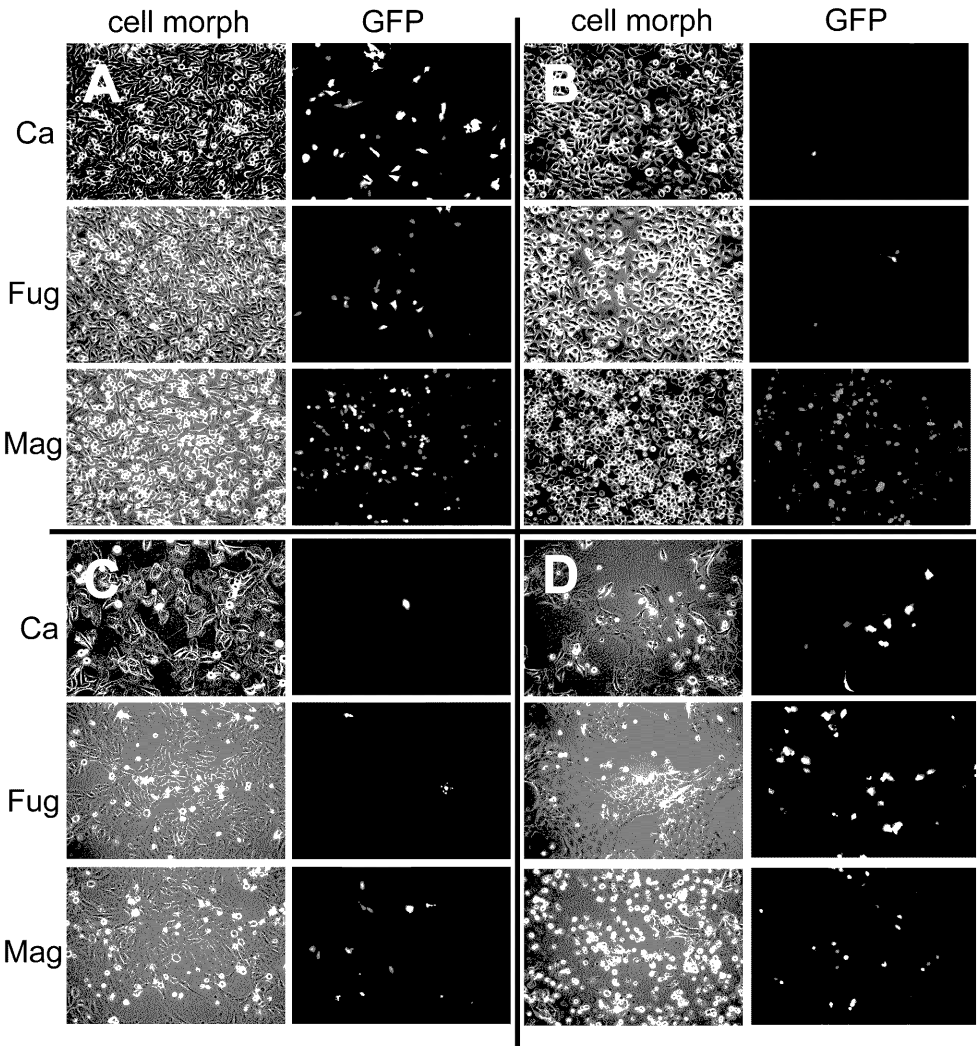


**Fig. 1.** Microscopic determination of transfection efficiency. pEGFP vector was transfected into HeLa cells (A), KB (B), YD-15 (C), and YD-38 (D). After 24 hours of transfection, cells onto dish were investigated under the fluorescence microscope. Ca, cells transfected by using calcium ion; Fug, cells transfected by chemical transfection reagent, FuGene6; Mag, cells transfected by magnetofection.

attached onto the culture dishes were examined under the fluorescence microscope as previous mentioned in Materials and Methods. The green signal of cells means that cells are transfected with vector DNA and are expressing the green fluorescence protein properly. In case of the simple transfection by using HBS-calcium ion, cells with the green signal were less than 10% average in total cell population (10% in HeLa, 1-2% in KB and YD 15, 5% in YD 38) (Fig. 1, upper panels in A-D). In case of using the chemical transfection reagent, FuGene 6, cells transfected were about 15% average (10% in HeLa, 1-2% in KB and YD 15, 5% in YD 38) (Fig. 1, middle panels in A-D). Surprisingly, by using the mignetofection nanoparticle, most of HeLa and KB cells were containing the vector DNA and were expressing green fluorescence protein. In case of other oral cancer cells, YD 15 and YD 38, more than 30% of cells were transfected, and detected as green signals under the microscope (Fig. 1, lower panels in A-D).

To determine the amount of green fluorescence protein expressed in cells, we then performed the Western analysis with crude cell extract by using anti-GFP antibody. Cells were collected after 24 hours of transfection. Total proteins of cell extract (50 μg) were separated by 10% SDS-PAGE and transferred onto a PVDF membrane. The amount of green fluorescence protein increased in cells transfected by magnetofection method, suggested that the result of Western analysis is coincided with the intensity of green signal (Fig. 2, lanes 3 in A-D). As expected, the green fluorescence protein levels from cells transfected by calcium method and transfectant reagent were lower than that from cells transfected by magnetofection (Fig. 2, lanes 1 & 2 in A-D). In these data, magnetofection did not seem to be effective on transfection in YD-15 cells (Fig. 1 C and Fig. 2 C). However, because of its high efficiency of gene delivery in most of the cell lines, magnetofection technique may be a powerful tool for target gene delivery into cells.

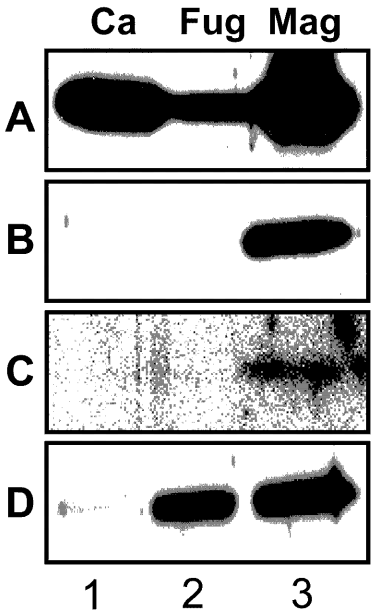


**Fig. 2.** Expression level of green fluorescent protein from cell extract. pEGFP vector was transfected into HeLa cells (A), KB (B), YD-15 (C), and YD-38 (D). After 24 hours of transfection, cells were collected and lysed for cell extract. Green fluorescence proteins expressed were detected by anti-GFP antibody as described in Materials and Methods. Lanes 1, proteins from cells transfected by using calcium ion (Ca); lanes 2, proteins from cells transfected by chemical transfection reagent, FuGene6 (Fug); lanes 3, proteins from cells transfected by magnetofection (Mag).

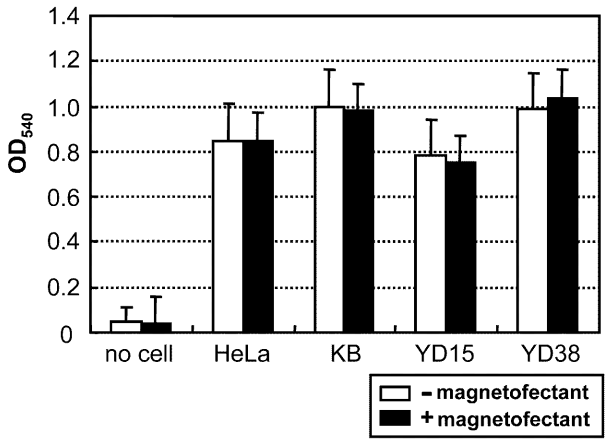


**Fig. 3.** Effect of nanoparticles used in magnetofection on cell viability. Cells were incubated with nanoparticles (PolyMAG$^{TM}$) for magnetofection for 30 hours. Viable cells were quantified by MTT assay as described in Materials and Methods. Solid bars, MTT signals from cells incubated with magnetofectant; empty bars, MTT signal from cells without magnetofectant.

To confirm whether the nanoparticles used in magnetofectioned cell have an effect on the cell viability, we performed the cell viability assay with MTT reagent. For this experiment, cells were treated with PolyMAG$^{TM}$ nanoparticle for 30 hours without DNA, and MTT reagent was treated and evaluated (see Materials and Methods). As shown in Fig. 3, cells treated without or with PolyMAG$^{TM}$ particles have no differences between their MTT uptakes, meaning that these nanoparticles have no effect on cell viability. Finally, in this study, we showed that magnetofection technique enhances transfection efficiency of the gene of green fluorescence protein (pEGFP) in several cell lines.

## Discussion

So far, very low efficiency of target gene expression has been reported in oral carcinoma cells such as human mouth epidermal carcinoma (KB), tongue mucoepidermoid carcinoma (YD-15), and squamous cell carcinoma (YD-38). Therefore, the magnetofection method for gene delivery may be of great use for investigating protein function in cell lines, which have difficulties in transfection, especially in oral cells.

In this report, we showed that magnetofection is an efficient technique for gene delivery in oral cancer cells. Numerous modifications of gene transfer tools have been designed to improve the effective gene delivery in human cells. Magnetofection as a new transfection technique to accumulate gene delivery complexes on the target tissue by exploiting magnetic forces was introduced by Scherer and colleagues (2002). In addition, Soeren *et al.* (2004) proposed that magnetofection was shown to efficiently transfer permanent airway epithelial cells. To address the potential of magnetofection of oral cells, which could not be transfected efficiently, 3 different kinds of oral carcinoma cells were transfected by using calcium ion and transfection reagent as well as magnetofection particles. Their transfection efficiencies were investigated under fluorescence microscopy and analyzed by Western blot. In most of the cell lines, magnetofection was more effective than other transfection reagents (Fig. 1 and 2). Especially, it is an effective tool for transfection of epithelial cells (KB cell, YD-15 cell, and YD-38 cell) allowing high efficiencies of gene transfer. Moreover, the ingredients used in magnetofection were no harm in cell viability (Fig. 3). This data suggest that magnetofection could be a good substitute for non-invasive gene transfer, which is simple, fast, and efficient in most types of cells. Therefore, it may be of great use for investigating protein functions in primary and cancer cells. Recently, we investigated that Polo-like kinase-1, one of the mitotic kinases, was highly expressed in oral cancer cells and gingival fibroblast by using the magnetofectant technique successfully (Ji and Jang, 2007). In conclusion, magnetofection, which may overcome fundamental limitations of gene transfer, is efficient tool for gene delivery, for investigating protein function in vivo, and for therapeutic application for drug targeting.

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