

## Optimization of SW480 Colon Cancer Cells Transfection with Lipofectamine 2000

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**ABSTRACT**

**Background and Objectives:** Nonviral carriers including those based on synthetic cationic lipids, offer several advantages over the viral counterparts. These carriers are able to form complexes with nucleic acids and deliver genes into the cells via the cellular endocytosis pathway, without significant toxicity. The level of transgenes expression depends on some experimental variables including cell type and density, Lipofectamine and DNA concentrations and Lipofectamine-DNA complexing time. The main objective of this study was to optimize transfection of SW480 colon cancer cells with Lipofectamine 2000.

**Methods:** In this study, SW480 cells were transfected with plasmid containing green fluorescent protein reporter gene using Lipofectamine 2000. Green fluorescent protein expression was studied under a reverse fluorescence microscope and the results were analyzed with the ImageJ software. Effect of different quantities of plasmid DNA and different Lipofectamine 2000 volumes on cell transfection efficiency was evaluated.

**Results:** The optimal volume of Lipofectamine and quantity of plasmid was 2  $\mu$ l and 1 $\mu$ g, respectively, which showed 59% efficiency for the transfection of SW480 cells at 24 hours post-transfection.

**Conclusion:** This study shows that Lipofectamine 2000 is an efficient reagent for the delivery of genes into SW480 cells. According to the results, the quantity of DNA per transfection and reagent concentrations are essential factors for a successful transfection.

**Keywords:** Optimization; pEGFP-N1; Lipofectamine; SW480.

## INTRODUCTION

Delivery of nucleic acids into eukaryotic cells is an appropriate method of studying gene expression. Several techniques have been described for the delivery of nucleic acids into different cell lines. Retroviral, lentiviral or adenoviral carriers are widely used for the transfer of genes into many eukaryotic cells (1, 2). These carriers are able to efficiently carry and express exogenous proteins. However, high cost and biosafety issues of retroviral and lentiviral vectors have limited their applications (1, 3). The use of non-viral carriers including those based on synthetic cationic lipids can resolve the limitations of viral carriers. These carriers are able to form a complex with the nucleic acid (lipoplex) and deliver genes into the cells via the cellular endocytosis pathway, without significant toxicity (4, 5). Cationic liposomes have many advantages over the viral carriers including excellent safety profile and immunogenicity and ease of use for large-scale production (6). The inability to integrate into the host genome is a particularly attractive aspect of cationic liposomes, which minimizes risk of mutagenic events in the transformed cell (7). Under physiological conditions, nucleic acids and cell surface membrane are negatively charged. A cationic reagent is required for the successful delivery of nucleic acids into cells. For cell transfection, the ability of cationic liposomes containing multivalent lipid (e.g. Lipofectamine) is higher than that of monovalent lipid-containing liposomes such as lipofectin (9, 10). However, they all have common features including: 1) a positively charged head group usually consisting of one or more nitrogen atoms, which induces an interaction between the transfection reagent and sugar-phosphate molecules of nucleic acid; 2) presence of a spacer that links the head group to one, two or three hydrocarbon chains. In some cases, the spacer may play a role in promoting contact between cationic lipids and nucleic acids (8, 9).

Some experimental variables influence the transfection efficiency. The cell type and Lipofectamine and DNA concentrations are important factors that need to be considered. The main objective of this study is to optimize or optimize transfection of SW480 colon cancer cells with Lipofectamine 2000.

## MATERIAL AND METHODS

Green fluorescent protein expression vector, pEGFP-NI (Clontech Laboratories, USA), was transformed into competent *Escherichia coli* strain DH5- $\alpha$  cells by heat shock method and grown under kanamycin treatment. After bacterial growth, plasmids were extracted using the Plasmid MidiPrep Purification Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. To confirm presence of plasmids, the extracted products were electrophoresed on 0.8% agarose gel.

Colorectal cancer SW480 cell line (Pasteur Institute, Iran) was cultured in RPMI1640 (Roswell Park Memorial Institute medium) supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/ml), and streptomycin (100  $\mu$ g/ml) (Gibco, UK). The cells were grown at 37 °C and in 5% CO<sub>2</sub>. Next, 2 $\times$ 10<sup>5</sup> cells were seeded onto a 24-well plate containing the aforementioned medium until the cells reached approximately 80% confluency at the time of transfection. Different densities of plasmid and different volumes of Lipofectamine were used to optimize the transfection of SW480 cells. For this purpose, 1-3  $\mu$ g of plasmid DNA and 1-3  $\mu$ l of Lipofectamine 2000 transfection reagent (Invitrogen, USA) were separately diluted with 50  $\mu$ l of RPMI-1640 medium without serum and the antibiotic. The mixture was incubated at room temperature for 30 minutes. Subsequently, the prepared complexes were added to the cells and incubated for 6 hours at 37 °C in 5% CO<sub>2</sub>. Then, the lipoplex-containing medium was aspirated and replaced with growth medium containing 5% FBS.

At 24 and 48 hours post-transfection, the cells were irradiated with UV (at wavelength of 450 to 490 nm). Green spots of GFP were observed and an image was randomly taken from the cells in three regions per well using a reverse fluorescence microscope (Olympus BX51, London, UK). The fluorescent images were analyzed with ImageJ software. Percentage of transfected cells was calculated based on the following formula:

$$\begin{aligned} & \text{Transfected cells (\%)} \\ &= \frac{\text{number of transfected cells}}{\text{Total number of cells}} \times 100 \end{aligned}$$

Transfection efficiency in relation to a serially diluted reagent and transferring vector was

analyzed using ANOVA and t-test in Microsoft Excel (2010). P-value <0.05 was considered as statistically significant.

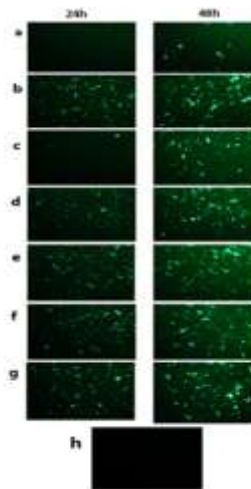
## RESULTS

We analyzed the efficiency of SW480 cells transfection with different quantities of plasmid and Lipofectamine 2000. Figure 1 shows the fluorescence expression profiles in GFP-transfected SW480 cells with different volumes of Lipofectamine 2000. Percentage of the transfected cells was compared for different transfection conditions at 24 hours and 48 hours post-transfection (Table 1). The data were normalized and analyzed with ANOVA and t-test (Figure 2). All quantities of plasmid showed significant efficiency. Transfection efficiency increased by increasing the amount of plasmid DNA, and the highest efficiency was recorded for 3 $\mu$ g plasmid. However, there was no statistically significant difference in the transfection efficiency when using different amounts of

plasmid DNA. For all amounts of plasmid used, transfection efficiency was higher at 48 hours post-transfection. At 24 hours post-transfection, the cells transfected with 1  $\mu$ l Lipofectamine and different amounts of plasmid did not show any significant fluorescence activity. However, increasing the volume of Lipofectamine increased the transfection efficiency, but this increase was not statistically significant. Furthermore, 1 $\mu$ g plasmid and 2  $\mu$ l Lipofectamine were optimal values for the transfection of SW480 cells because they were the lowest amounts of variables that showed significant transfection efficiency (59%).

Compared to 24 hours post-transfection, the transfection efficiency increased by 2-3 folds at 48 hours post-transfection and all concentrations of plasmid showed significant efficiency (Table 1). At 48 hours post-transfection, approximately 48% of cells were transfected when using 1 $\mu$ l Lipofectamine and 1 $\mu$ g plasmid.

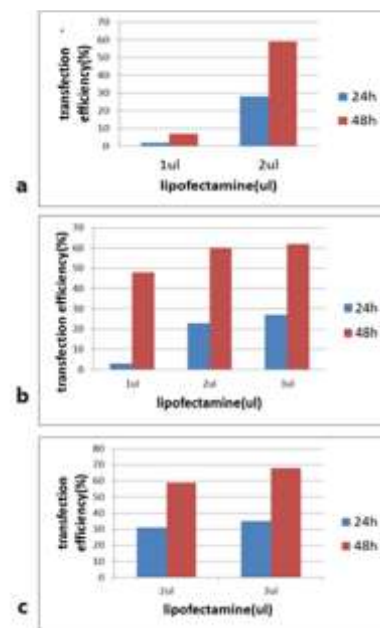
**Figure 1- Fluorescence expression profiles in GFP-transfected SW480 cells with different amounts of plasmid and Lipofectamine 2000 after 24 and 48 hours. A: 1  $\mu$ g plasmid + 1 $\mu$ l lipofectamine; B: 1 $\mu$ g plasmid + 2 $\mu$ l lipofectamine; C: 2 $\mu$ g plasmid + 1 $\mu$ l lipofectamine; D: 2 $\mu$ g plasmid + 2 $\mu$ l lipofectamine; E: 2 $\mu$ g plasmid + 3 $\mu$ l lipofectamine; F: 3 $\mu$ g plasmid + 2 $\mu$ l lipofectamine; G: 3 $\mu$ g plasmid + 3 $\mu$ l lipofectamine; H: untransfected cells.**



**Table 1- Percentage of GFP-transfected SW480 cells with different amounts of Lipofectamine 2000 at 24 and 48 hours post-transfection**

		24h			48h		
		Plasmid			Plasmid		
Lipofectamine		1 $\mu$ g	2 $\mu$ g	3 $\mu$ g	1 $\mu$ g	2 $\mu$ g	3 $\mu$ g
	1 $\mu$ l	2%	3%	--	7%	48%	--
	2 $\mu$ l	28%	23%	31%	59%	60%	59%
	3 $\mu$ l	--	27%	35%	--	62%	68%

Figure 2- Transfection efficiency for different amounts of plasmid. A:1 µg plasmid. B:2 µg plasmid. C:3 µg plasmid



## DISCUSSION

Several mechanisms have been reported for the delivery of nucleic acids into eukaryotic cells. Viral carriers are highly efficient but also limited due to various safety issues and risk of neoplasia and infectivity. Nonviral reagents resolve some of these limitations but at the cost of efficiency and higher cellular toxicity. The efficiency of nonviral reagents is variable in vitro and depends on the cell type. These reagents are cationic, form a complex with the negatively charged nucleic acids, and cell surface membrane. The complexes enter the cell through clathrin- and caveolae-mediated endocytosis (11, 12). The goal of this study was to determine the efficiency and optimal conditions for gene delivery into human colorectal SW480 cells using the Lipofectamine 2000 reagent. We found that all concentrations of plasmid show considerable efficiency with no significant difference between the efficiency of each concentration. Moreover, 1 µg plasmid and 2 µl Lipofectamine were determined as the optimized amounts of variables for the transfection of SW480 cells. Transfection efficiency was higher with all quantities of plasmid at 48 hours post-transfection compared to 24 hours post-transfection. In a study by Ward and Stern, lipofection of pEGFP-N1 plasmid and CMV promoter caused

more than 50% GFP expression in five different murine cell lines (13). Shabani et al. transfected pEGFP-N1 vector in five murine myeloma cell lines using LyoVac, jetPEI, and Lipofectamine 2000. They reported that the transfection of cells with Lipofectamine 2000 had higher efficiency compared to other reagents (14). Salimzodeh et al. transferred GFP vector in lung cancer cell line using CaP, DEAE-dextran, superfect, electroporation, and lipofection methods. In the mentioned study, Lipofectamine 2000 demonstrated the highest efficiency (40.1%) for the lipofection of Mehr-80 cell line (15).

Hashemi et al. compared efficiency of transferring pEGFP-N1 vector into Huh-7 and Vero cells with electroporation, transfection by Lipofectamine 2000, and jetPEI. They reported that Lipofectamine 2000 with efficiency of 63% and 73% was the most suitable reagent for transferring the plasmid into the Huh-7 and Vero cells, respectively (16).

## CONCLUSION

This study shows that Lipofectamine 2000 is an efficient reagent for the delivery of genes into SW480 cells. According to the results, the quantity of DNA per transfection and reagent concentrations are essential factors for a successful transfection.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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