Optimization of TransFectin™ Lipid Reagent-Mediated Transfection for Different Cell Types

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Introduction

The ability to introduce DNA into cells provides a powerful tool for studying the in vivo function and control of mammalian genes. Methods and reagents designed for nucleic acid delivery have received considerable attention, especially in protocols and discussions related to effective gene therapy strategies.

TransFectin lipid reagent is a mixture of a proprietary cationic lipid and colipid DOPE (1,2-dioleoylphosphatidylethanolamine). Cationic lipids, brought in contact with aqueous solutions under special conditions, form positively charged micelles or liposomes. These micelles associate with the negatively charged phosphates of nucleic acids and form spontaneous complexes with DNA or RNA. The lipid-DNA (or -RNA) complexes fuse or associate with the cell membrane via hydrophobic and electrostatic interactions, and the complex is then internalized (Zhou and Huang 1994, Remy et al. 1994).

It is important to optimize transfection conditions not only for each transfection method but also for every cell type. Cell density, duration of transfection, volume of medium during transfection, and ratio of lipid reagent to DNA are key factors for efficiency. In this article we describe the optimization of transfection with TransFectin monitored by a secreted alkaline phosphatase (SEAP) reporter gene assay. We compare two suspension cell types that are considered difficult to transfect: bovine peripheral blood mononuclear cells (PBMCs) and bovine B-lymphosarcoma BL3.1 cells (Anderson et al. 2004, Fenton et al. 1998). Additionally, we optimize the transfection efficiency for two adherent cell types: primary bovine oviductal cells and a calf pulmonary artery endothelial cell line (CPAE).

Methods

Cell Cultures

Lymphocytes were prepared from heparinized bovine peripheral blood after 1:1 dilution with PBS and Ficoll-Paque PLUS (Amersham Biosciences) density gradient centrifugation. Cells were cultured in RPMI-1640 medium (supplemented with 25 mM glucose, 10 mM HEPES, 1 mM sodium pyruvate, and 10% FCS). The cell density was adjusted to 4 x 10^6 cells/ml. PBMCs were transfected after 20–24 hr of cultivation. The B-lymphosarcoma cell line, BL3.1, was obtained from ATCC (CRL-2306). Cell density of suspension cells BL3.1 was adjusted to 5 x 10^5/ml 1 day prior to transfection in RPMI-1640 medium.

Bovine oviductal cells were freshly prepared as described previously (Ulbrich et al. 2003) with some modifications: After centrifugation (550 x g for 5 min), 50 µl cell pellet was diluted in 20 ml Medium 199 (supplemented with 20 mM HEPES, 0.68 mM sodium pyruvate, 2.3 mM L-glutamine, and 10% FCS). This preparation was designated the initial dilution, and further dilutions were prepared for optimization of cell density. The cultivated primary cells were transfected 1 day after they became adherent (4 days after preparation). The CPAE cells (ATCC, CCL-209) were plated in a concentration of 5 x 10^4 cells/ml in MEM-Earle (supplemented with 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 mM glutamine, and 20% FCS) 1 day before transfection.

Each cell type was seeded on 24-well culture plates in 1 ml of the appropriate growth medium per ml and incubated at 37°C in a humidified 4.5% CO_2 atmosphere. The cell viability and proliferation were surveyed by colorimetric assay using WST-1 cell proliferation reagent (Roche) as recommended by the manufacturer.

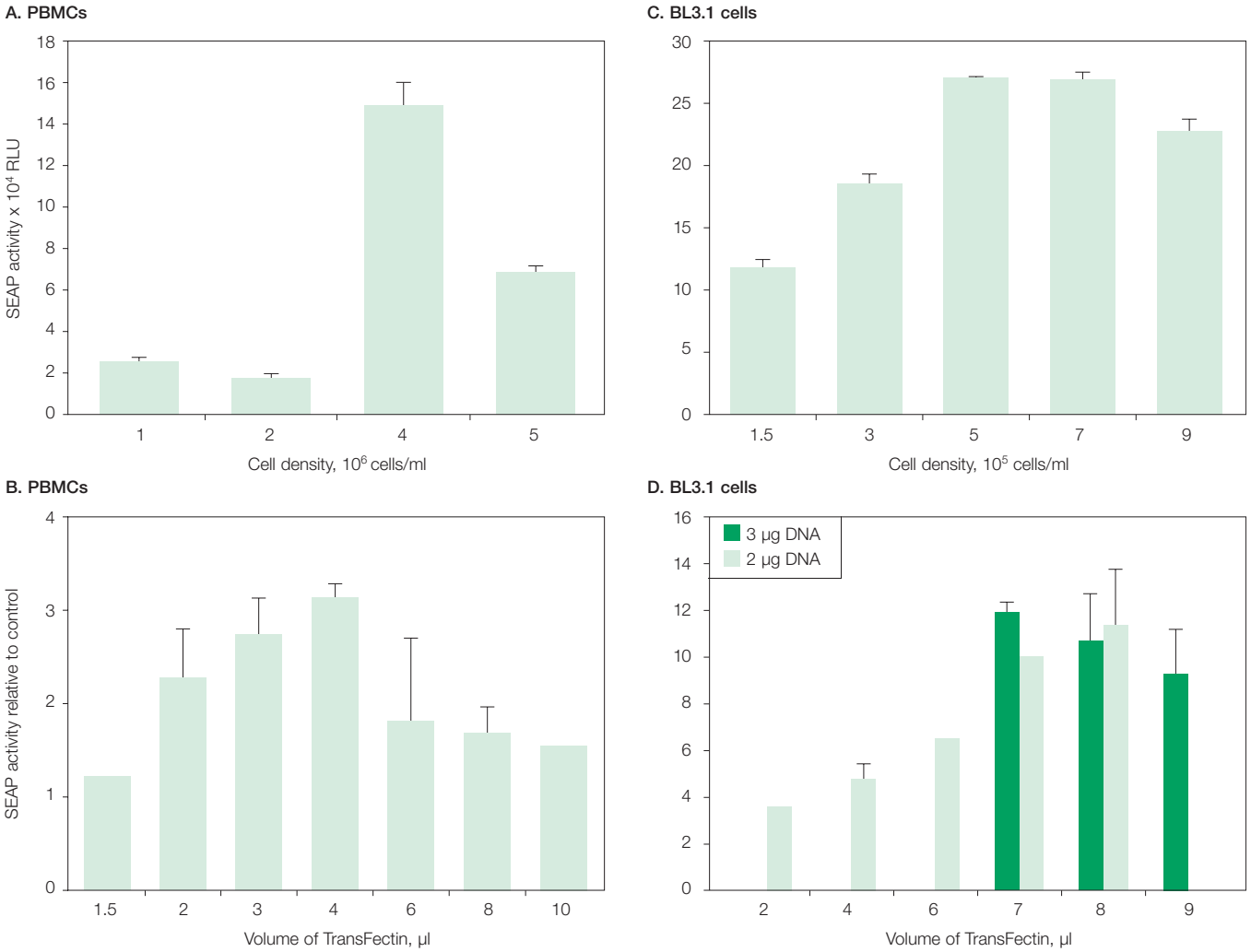

Fig. 1. Optimization of transfection conditions for suspension cells. Conditions were optimized for PBMCs (A, B) and BL3.1 cells (C, D). To evaluate the effect of cell density on transfection efficiency, cells were seeded at indicated concentrations 24 hr prior to transfection using 2 µg DNA (pTAL-SEAP2-control) and 4 µl (A) or 7 µl (C) TransFectin. To evaluate the effect of the amount of TransFectin on transfection efficiency, PBMCs (4 x 10^6/ml) were transfected using 2 µg DNA (pTAL-SEAP2-control) and indicated amounts of TransFectin (B). BL3.1 cells (5 x 10^5/ml) were transfected using 2 or 3 µg DNA (D). Cell culture supernatant was assayed 24 hr after transfection for SEAP activity. Values are expressed relative to those for transfection with a promoterless control plasmid (error bars indicate standard deviation; A, n = 4; B, n = 6; C, n = 4; D, n = 7).

Plasmids

Cells were transfected with the reporter plasmid TAL-SEAP2-control (Clontech), which contains the SEAP gene under control of the SV40 early promoter/enhancer or with a promoterless plasmid as negative control. In some cases cells were additionally transfected with a vector containing a β-galactosidase reporter gene under the control of the CMV promoter (CMVβ-Gal, kindly provided by Grant R MacGregor, Stanford University, USA).

Transfection Protocol for 24-Well Plate Format

For each well, DNA was diluted in 50 µl of serum-free growth medium and mixed gently briefly. TransFectin was prepared in 50 µl of serum-free growth medium per ml. Both solutions were combined, mixed gently, and incubated at room temperature for 20 min to allow TransFectin-DNA complex formation. The TransFectin-DNA mixture (100 µl) was added to the growth medium in each well and mixed gently by rocking the plate. Cells were incubated at 37°C and 4.5% CO_2.

Detection of Transfection Efficiency

The relative transfection efficiency was assessed by measuring SEAP activity in the culture media using a chemiluminescent SEAP reporter gene assay (Roche) according to the manufacturer's protocol. For quantitation, 25 µl of the transfected cell culture supernatant was used. The resulting light signal was measured in a Sirius luminometer (Berthold). Values were expressed in relative light units (RLU) or as relative to the value from cells transfected with a promoterless plasmid. The β-galactosidase activity was estimated using a histochemical kit (Roche). Cells were stained with X-Gal 2 days after transfection.

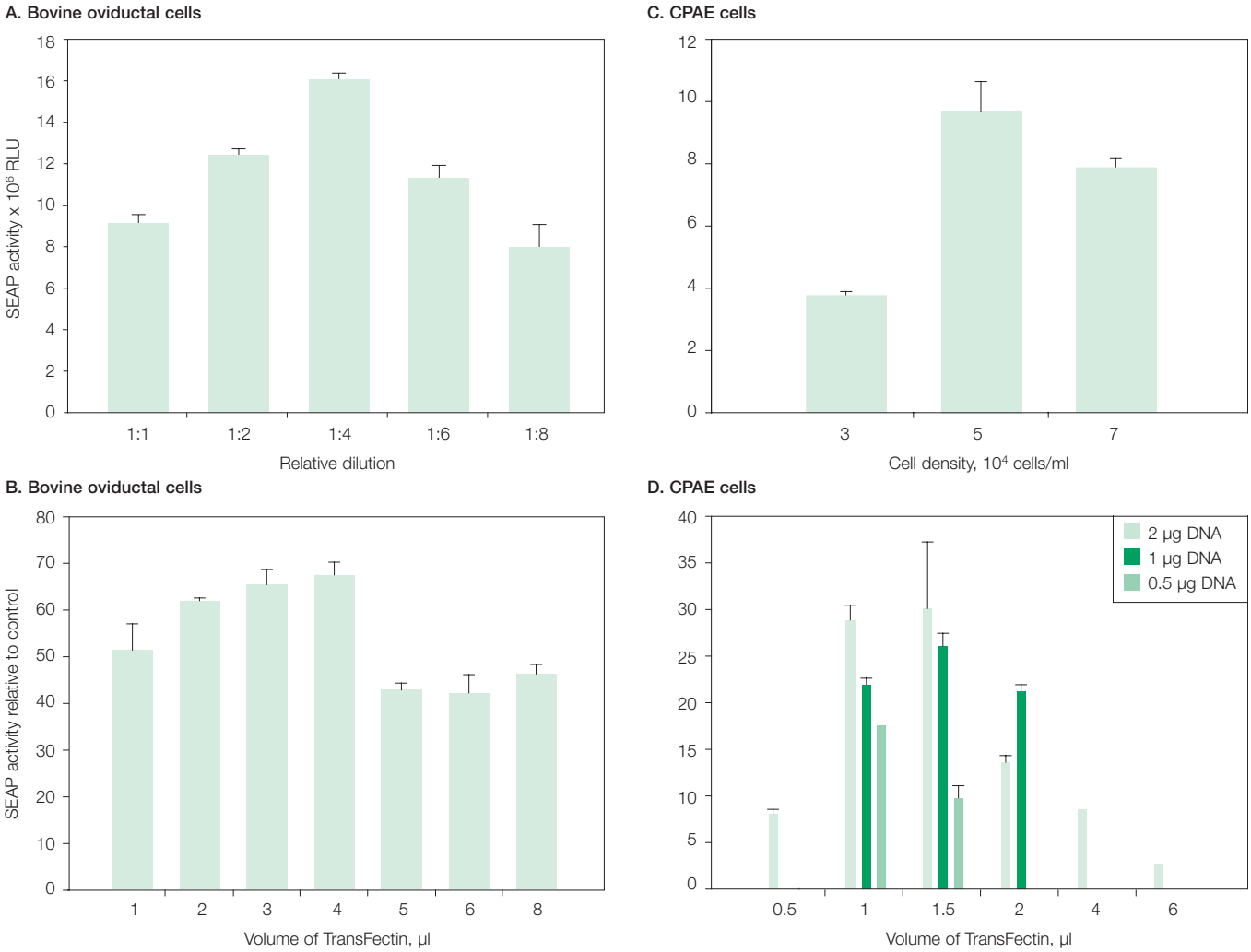

Fig. 2. Optimization of transfection conditions for adherent cells. Conditions were optimized for primary bovine oviductal cells (A, B) and CPAE cells (C, D). To evaluate the effect of cell density on transfection efficiency, primary bovine oviductal cells (A) were seeded at different dilutions of the initial cell suspension (a 1:4 dilution of the initial cell suspension prior to seeding corresponds to a cell density of 3–5 x 10^4 cells/ml in a 4 days' culture) and transfected using 2 µg DNA (pTAL-SEAP2-control) and 4 µl TransFectin. CPAE cells (C) were seeded at the indicated concentrations 24 hr prior to transfection using 2 µg DNA and 2 µl TransFectin. To evaluate the effect of the amount of TransFectin on transfection efficiency, primary bovine oviductal cells (3–5 x 10^4/ml) were transfected using 2 µg DNA (pTAL-SEAP2-control) and indicated amounts of TransFectin (B). CPAE cells (5 x 10^4/ml) were transfected using 0.5, 1, or 2 µg DNA (D). Cell culture supernatant was assayed 24 hr after transfection for SEAP activity. Values are expressed relative to those for transfection with a promoterless control plasmid (error bars indicate standard deviation; A, n = 4; B, n = 9; C, n = 4; D, n = 5).

Results

Suspension Cultures

Transfection conditions were optimized for lymphoid cells (PBMC and BL3.1). TransFectin/DNA ratio as well as cell density are known to affect transfection efficiency. Therefore, we examined in initial experiments the impact of cell density for each cell type, and then optimized the TransFectin/DNA ratio. With PBMCs, the highest SEAP activity was achieved by seeding 4 x 10^6 cells/ml 24 hr prior to transfection (Figure 1A). Figure 1B shows the results of TransFectin/DNA ratio optimization for PBMCs. The highest efficiency was achieved with 2 µg DNA and 4 µl TransFectin. Neither 1 µg nor 3 µg DNA resulted in higher efficiency on PBMCs at all tested TransFectin/DNA ratios (data not shown).

Similar experiments were carried out with BL3.1 cells in suspension. For these cells, the best results were obtained with 5–7 x 10^5 cells/ml (Figure 1C). In comparison to the PBMCs, the established B-lymphosarcoma cell line, BL3.1, showed higher transfection efficiencies. The best results were achieved using 2–3 µg DNA and 7–8 µl TransFectin (Figure 1D).

Adherent Cells

Primary bovine oviductal cells revealed highest transfection efficiencies at a cell density of 3–5 x 10^4 adherent cells per ml (4 days' culture). This corresponded to a 1:4 dilution of the initial cell suspension prior to seeding (Figure 2A). As shown in Figure 2B, the best TransFectin/DNA ratio for this cell type was 4 µl/2 µg. Furthermore, CPAE cells can be transfected

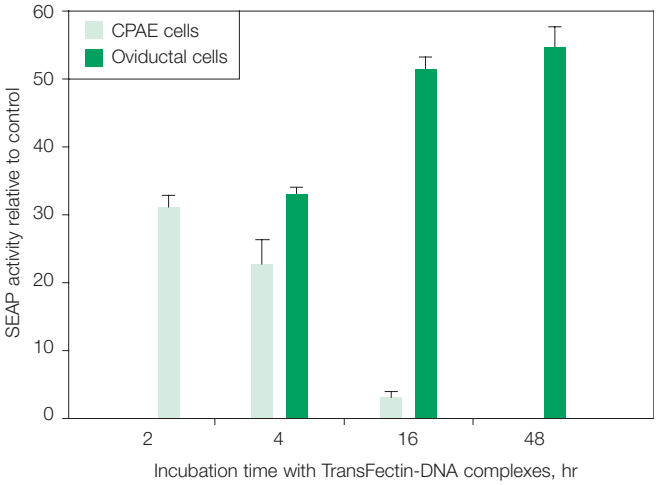

Fig. 3. Effect of incubation time on transfection efficiency of CPAE and bovine oviductal cells. CPAE cells (5 x 10^4/ml) were transfected with 1 µg DNA (pTAL-SEAP2-control) using 1.5 µl TransFectin, and bovine oviductal cells (3–5 x 10^4/ml) with 2 µg DNA and 4 µl TransFectin. Medium was replaced after indicated incubation times of cells with TransFectin-DNA complexes. Cell culture supernatant was assayed 24 hr (CPAE cells) or 48 hr after transfection (oviductal cells) for SEAP activity (error bars indicate standard deviation; n = 3).

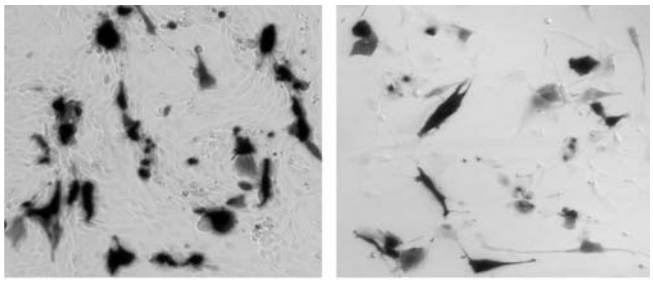

Fig. 4. Expression of β-galactosidase. Left, 3–5 x 10^4/ml bovine oviductal cells transfected with pCMVβ-Gal using 2 µg DNA and 4 µl TransFectin; right, CPAE cells transfected using 1 µg DNA and 1.5 µl TransFectin. β-Galactosidase activity was visualized 48 hr after transfection by histochemical staining.

efficiently using a cell density of 5 x 10^4/ml (Figure 2C). Comparable results were obtained with 1–1.5 µl TransFectin and 1–2 µg DNA (Figure 2D). This cell line was very sensitive to TransFectin-DNA complexes. In contrast to primary bovine oviductal cells, the removal of the complexes 2 hr after transfection was essential for CPAE cells (Figure 3). Additionally, a vector containing a β-galactosidase reporter gene under the control of the CMV promoter was introduced and confirmed our previous results (Figure 4).

Table 1. Recommended transfection conditions.

	PBMCs	BL3.1	Oviductal Cells	CPAE
Cell concentration	4 x 10^6/ml	5–7 x 10^5/ml	3–5 x 10^4/ml	5 x 10^4/ml
TransFectin/ DNA ratio	4 µl/2 µg	7 µl/2 µg	4 µl/2 µg	1–1.5 µl/1–2 µg
Addition or replacement of medium after transfection	Add after 4 hr	Add after 12 hr	Not needed	Replace after 2 hr
Duration of transfection	24 hr	24 hr	48 hr	24 hr

Conclusions

We evaluated transfection efficiency of bovine PBMCs, bovine B-lymphosarcoma cells, calf pulmonary aortic endothelial cells, and primary bovine oviductal cells using TransFectin lipid reagent. For the cell types tested, we recommend using a 24-well plate format under the transfection conditions detailed in Table 1. Remarkable transfection efficiencies were achieved by adjusting cell densities prior to transfection; quantities of TransFectin reagent and DNA; and incubation time of cells with TransFectin-DNA complexes. The results demonstrate the need for testing broad ranges of TransFectin/DNA ratios with each cell line in order to achieve maximum transfection efficiencies. TransFectin turned out to be highly efficient in these cell types, which are considered difficult to transfect.

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