Efficient Reverse Transfection of Both siRNA and Expression Plasmids on Transfected Cell Microarrays Using X-tremeGENE siRNA Transfection Reagent


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Introduction

Transfected cell microarray is a miniaturization of cell-based functional studies and allows spatially restricted transfection without the use of wells [1]. Expression plasmids and/or siRNAs complexed with a transfection reagent in a gel are printed on a glass slide. Cells growing on top of such printed spots will take up the nucleic acids deposited in the spot, while cells growing between the spots will not be transfected, resulting in overexpression or silencing of specific gene products in groups of living cells. By applying appropriate assays, phenotypic consequences of the gene perturbations can be detected (*e.g.*, genes involved in signal transduction and transcriptional regulation, apoptosis and subcellular localization can be identified) (Figure 1). The transfected cell microarray format has enormous possibilities when it comes to both high-throughput screening and more medium-scale functional studies. We have optimized the method for medium-scale functional studies with expression plasmids and siRNAs using X-tremeGENE siRNA Transfection Reagent [2]. Here we describe technical considerations of the method and show some examples of possible biological readouts.

Materials and Methods

Printing solution

The printing solution was optimized as described in the results and applications section, and the following composition gave high transfection efficiency and well-localized spots in HEK 293 cells: In a 1.5-ml microcentrifuge tube, plasmid (1 µg/µl) and siRNA were mixed with growth medium without fetal calf serum (FCS), 0.5 µl 1.5 M sucrose, and 3 µl X-tremeGENE siRNA Transfection Reagent per

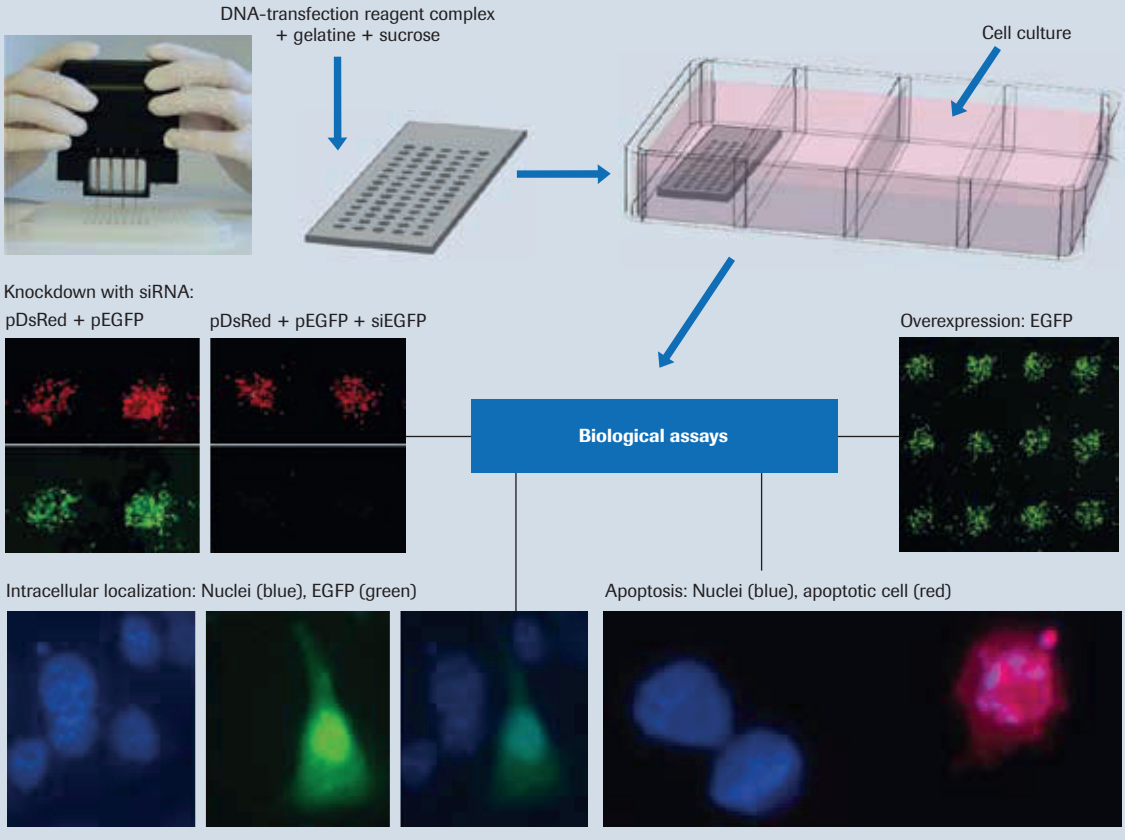

Figure 1: Workflow for transfected cell microarrays. Cells are added to the printed slides, and cells growing on top of the printed spots will be transfected. The phenotypic effect of the gene perturbation can be detected using different biological assays, such as fluorescent reporter genes, labeling of apoptotic cells and detection of intracellular localization by using fluorescently labeled gene products.

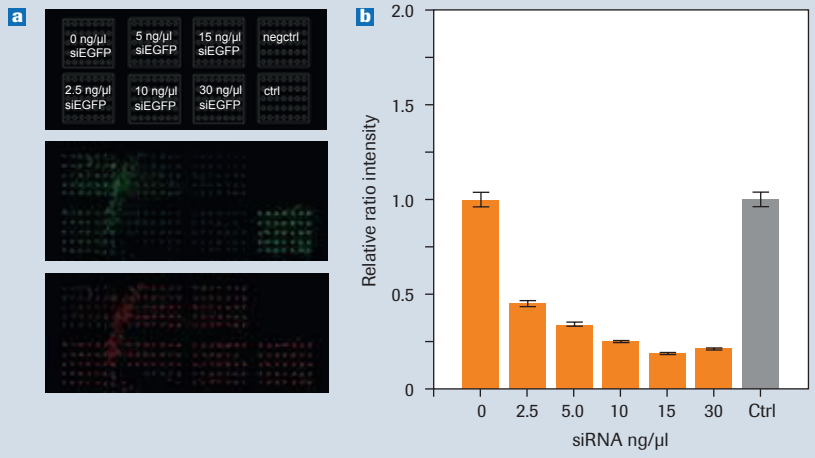

Figure 2: Transfection of HEK 293 cells with plasmid and siRNA. Cells were cotransfected with pEGFP (30ng/µl), pDsRed (30 ng/µl) and different concentrations of siEGFP (0-30 ng/µl). pCRE-Luc (60 ng/µl) was used as a control of the background fluorescence in the spots (negctrl). pEGFP, pDsRed and 30 ng/µl siCAT was used as an siRNA control (ctrl). **(a)** Top diagram: placement of the cell clusters for eight conditions. Middle diagram: scanning image for EGFP-detection. Bottom diagram: scanning image for DsRed-detection. **(b)** Estimated effects of the different conditions are shown based on the EGFP fluorescence intensity data normalized to DsRed control plasmid intensities. The intensities are shown relative to 0 ng/µl siRNA; error bars are 95% confidence interval. (Modified from [2] with permission from Nucleic Acids Research).

microgram nucleic acid to a final volume of 22.5 µl. After 15-20 minutes of incubation, 7.5 µl 0.4% gelatine (Type B, G9391, Sigma) was added to give 30 µl printing solution.

Array printing

The nucleic acid-lipid-gelatine solution was arrayed onto UltraGAPS™-coated slides (Corning) at room temperature. The arrays were printed using a 10-µl pipette tip (Biosphere Filter Tips, type Gilson/Biohit, Sarstedt) giving spots of about 800 µm in diameter, or using the hand-held microarrayer MicroCaster™ (Schleicher & Schuell), giving spots of about 500 µm in diameter (Figure 1).

Reverse transfection of HEK 293 cells

HEK 293 cells were cultured at 37°C in a humidified 5% CO_2 incubator in Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g/l glucose (Gibco, Invitrogen) supplemented with 1% (v/v) penicillin-streptomycin (Gibco, Invitrogen), 0.1 mg/ml L-glutamine (Gibco, Invitrogen), and 10% FCS (Euroclone).

Immediately prior to transfection, actively growing cells were trypsinized and resuspended in growth medium to desired density. The printed slides were placed in QuadriPERM plates (Vivascience) and overlaid with 3 x 10^6 cells in 8 ml medium. After 48 hours of incubation, the slides were gently washed in PBS, and the cells were fixed (3.7% paraformaldehyde and 4% sucrose in PBS) for 20 minutes at room temperature. After the slides had been washed, the nuclei were stained with DAPI (Invitrogen) and the slides mounted with Mowiol 4-88 (Hoechst).

Analysis and biological readouts

For analysis of spot intensities, transfected cell microarrays were scanned using Tecan's LS Reloaded™ scanner, and the level of fluorescent protein expression in each spot was quantified using the GenePix software (Axon Instruments). Linear regression (ANOVA) modeling was used for estimating biological effects and calculating p-values for comparison of interests based on data from several experimental replicates [2]. Apoptotic cells were detected using the In Situ Cell Death Detection Kit, TMR red according to the manufacturer's description. Intracellular localization of gene products was performed using EGFP-tagging. Apoptotic cells and intracellular protein localization were detected using a fluorescence microscope.

Results and Applications

Our protocol [2] is based on the so-called lipid-DNA method reported by Ziauddin and Sabatini [1]. The workflow includes making the printing solution, printing the arrays, incubating cells on the top of the arrays, and detecting the resulting effects in the spots.

The printing solution, which consists of nucleic acid(s), transfection reagent, sucrose and gelatine, has to be optimized in order to achieve high transfection efficiency, as well as spots with well-defined edges to avoid cross-contamination between spots. We first tested several transfection reagents (data not shown) and found that the X-tremeGENE siRNA Transfection Reagent yielded high transfection efficiency in HEK 293 cells for both siRNA and expression plasmid together, as well as for plasmid alone on transfected cell microarrays. Figure 2 shows the transfection of pEGFP and pDsRed with increasing concentrations of siEGFP.

Both the amount of transfection reagent and the concentrations of sucrose and gelatine influence transfection efficiency and spot integrity. Gelatine is added to the printing solution in order to facilitate the transfer of the nucleic acid from the glass surface and into the cells, while sucrose is added to stabilize the complex between the nucleic acid and the transfection reagent. We found that 3 µl X-tremeGENE solution per

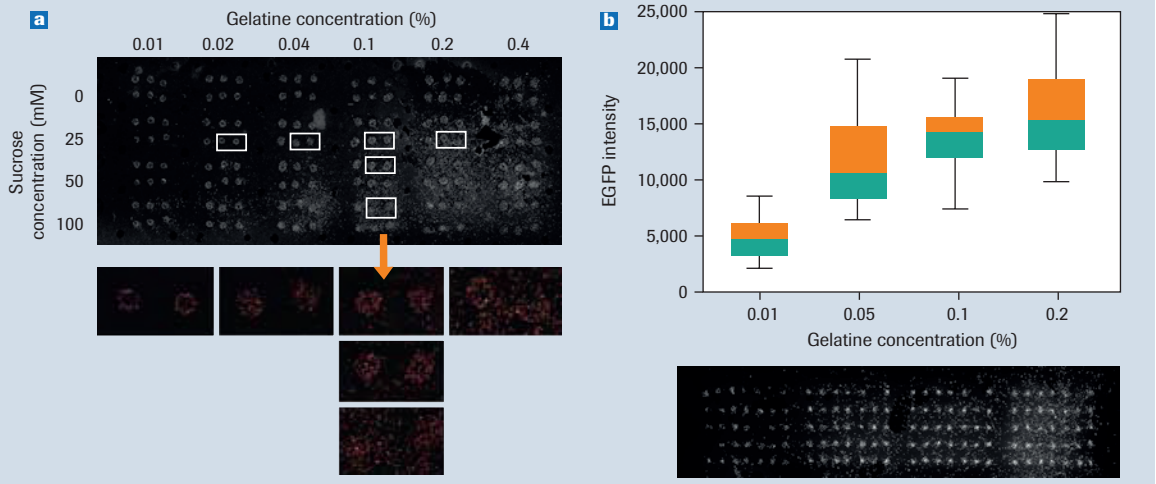

Figure 3: Effects of sucrose and gelatine concentrations on spot integrity and transfection efficiency. (a) Array printed with pDsRed (50 ng/µl) in a printing solution with different gelatine and sucrose concentrations. Scanning image of the whole array and magnifications of specific spots. **(b)** Array printed with pEGFP (50 ng/µl) in a printing solution with 25 mM sucrose and four different concentrations of gelatine. Top: Box plot of the fluorescence intensities in each spot (n=32-34). Bottom: Scanning image showing squares of seven times five spots for the four gelatine concentrations. From left to right: 0.01%, 0.05%, 0.1%, and 0.2% gelatine. (Reproduced from [2] with permission from Nucleic Acids Research).

microgramm nucleic acid gave good transfection efficiency. To achieve higher transfection efficiency, the amount of transfection reagent can be increased (in addition to adjusting the sucrose and gelatine concentrations). However, we have observed that too much X-tremeGENE may result in less localized spots (data not shown). Figure 3 shows representative images of the effects observed for different concentrations of gelatine and sucrose. We observed that the transfection efficiency increased with increasing gelatine concentration (tested in the range 0.01–0.40%). Addition of sucrose was observed to be especially beneficial for achieving high transfection efficiency when the printed slides were stored for several weeks prior to use (data not shown), and the transfection efficiency increased with increasing sucrose concentration (tested in the range 0–100 mM). However, an increased disturbance of the spatial definition of the spots was observed with the increasing gelatine or sucrose concentration. In addition, a combined effect of the concentrations of gelatine and sucrose was observed, as low concentrations of gelatine allowed us to use higher concentrations of sucrose than were possible with higher concentrations of gelatine before cells spread outside the spots (Figure 3a). Based on several optimizing experiments, we found that 3 µl X-tremeGENE solution per µg nucleic acid, 25 mM sucrose and 0.1% gelatine in the final printing solution reproducibly gave spatially restricted transfection with high transfection efficiency in HEK 293 cells (illustrated in Figure 3b).

For detecting the phenotypic effects of gene perturbations, a huge range of different biological readouts is possible. We have shown the potential of transfected cell microarrays for studying complex regulation of gene expression by enabling measurement of biological responses in cells with overexpression and downregulation of specific gene products, combined with the possibility of assaying the effects of external stimuli using fluorescent reporter genes as readouts [2]. Transfected cell microarrays can also be used to detect phenotypes such as apoptosis and intracellular localization (Figure 1).

Summary

Transfected cell microarray is a versatile, efficient, and cost-reducing technology [1,3,4] that can contribute to further understanding of gene functions and regulatory mechanisms governing gene expression. We have optimized the protocol of transfected cell microarrays using X-tremeGENE siRNA Transfection Reagent to transfect both expression plasmids and siRNAs, and have shown that this technology can be used to reliably detect even quantitatively minor biological effects of gene perturbations by including several technical and experimental replicates and using linear regression (ANOVA) modeling to estimate the biological effects [2]. ■

References

1. Ziauddin J, Sabatini DM (2001) Nature 411:107–110
2. Fjeldbo CS et al. (2008) Nucleic Acids Res 36:e97
3. Starkuviene V et al. (2007) Expert Rev Proteomics 4:479–489
4. Wheeler DB et al. (2005) Nat Genet 37 Suppl:S25-30

Order INFO

Product	Pack Size	Cat. No.
X-tremeGENE siRNA Transfection Reagent	1 ml (400 transfections in a 24-well plate)	04 476 093 001
	5 x 1 ml (2,000 transfections in a 24-well plate)	04 476 115 001