Effect of DNA Amount on Transfection-Related Cellular Toxicity

Application Note

Transfection is the process of introducing new nucleic acids into a cell. Quite often, the thought process in transfection is "more is better" – that is, the goal is to achieve the highest possible protein expression post-transfection. However, cells can be stressed by over-expression of specific proteins, especially when over-expression takes over the protein production machinery at the expense of producing other necessary proteins for the cell or by an innate immune response to CpG islands in DNA. In this application note, we examine the effect of DNA amount on transfection-related cellular toxicity.

Achieving a balanced transfection protocol

Transfection protocols are often written in a "one size fits all" approach, assuming that a single DNA concentration will work for the majority of cell lines. However, the reality is that different cell lines respond to transfection reagents with varying degrees of success, and that some cell lines are more sensitive than others to toxicity resulting from exposure to DNA, transgene overexpression, or innate immunity in response to CpG islands.

The data presented in this publication explores the balance between efficient DNA transfection and cellular health. As the data shows, cell lines respond differently to varying DNA amounts used in transfection protocols, suggesting that researchers need to consider both the transfection reagent and the amount of DNA used during transfection when performing this type of experiment.

Methods

Transfections were performed in 96-well flat-bottomed cell culture plates. Complexing reactions were performed in 96-well round-bottom plates. Cells were plated approximately 24 hours before transfection in 100 µl/well of complete medium, at a density optimized to reach ~70-80% confluence by the time of transfection.

Complexing reactions:

DNA was diluted in a final volume of 100 µl of complete medium to 0-20 µg/ml DNA for either GFP or β-galactosidase. 0.5-5 µl of DNA-In® transfection reagent (MTI-GlobalStem, catalog #73750) was added per 100 µl diluted DNA (Table 1).

After thorough mixing, complexes were allowed to form at room temperature for 15 minutes.

Table 1. Complexing reactions in 100 µl

DNA ng/µL	20						10						5						2.5						1						0					
DNA-In® µL	0.5	1	2	3	4	5	0.5	1	2	3	4	5	0.5	1	2	3	4	5	0.5	1	2	3	4	5	0.5	1	2	3	4	5	0.5	1	2	3	4	5

Transfections:

Following the complexing incubation, 10 µl of complex was transferred to the appropriate wells of cells containing 100 µL media. Incubation was continued for 48 hours with no media change. After the 48 hour transfection period, cells were assessed for:

- GFP levels – measured using a Tecan fluorescent plate reader
- β-galactosidase levels – measured using an ONPG-based colorimetric assay at 405 nm on a Bio-Rad optical plate scanner

- Cellular toxicity – assessed using the Promega CellTiter 96® AQueous One Solution Cell Proliferation Assay
- Photomicroscopy - Transfections were examined visually, both under fluorescent and phase contrast illumination, to estimate percent cell transfection levels and confirm visual evidence of toxicity. Photos were taken of a representative field, both under UV and phase contrast, of the peak activity wells within each DNA concentration as determined by the Tecan scan (above).

All data was collected 48 hours post-transfection. Bar graphs show the expression level of either GFP or β-galactosidase with increasing concentrations of transfection reagent, from left to right. Line graphs represent the viability data from the same well as depicted in the corresponding bar graph. Viability data is normalized to untransfected cells.

Results

Sensitive cell lines and primary cells may require lower amounts of DNA during transfection

Most commercially available transfection protocols recommend using 10 µg/ml DNA for transfection experiments, which is equivalent to the 100 ng/well used in our experiments. However, some cell lines exhibit significant toxicity at this DNA concentration. For example, introduction of DNA for GFP (Figure 1A) caused significant toxicity at 10 µg/ml DNA in HeLa cells. Similar data was observed for β-galactosidase (data not shown). Toxicity was not due to the transfection reagent, as indicated by the transfection reagent only data (Figure 1A).

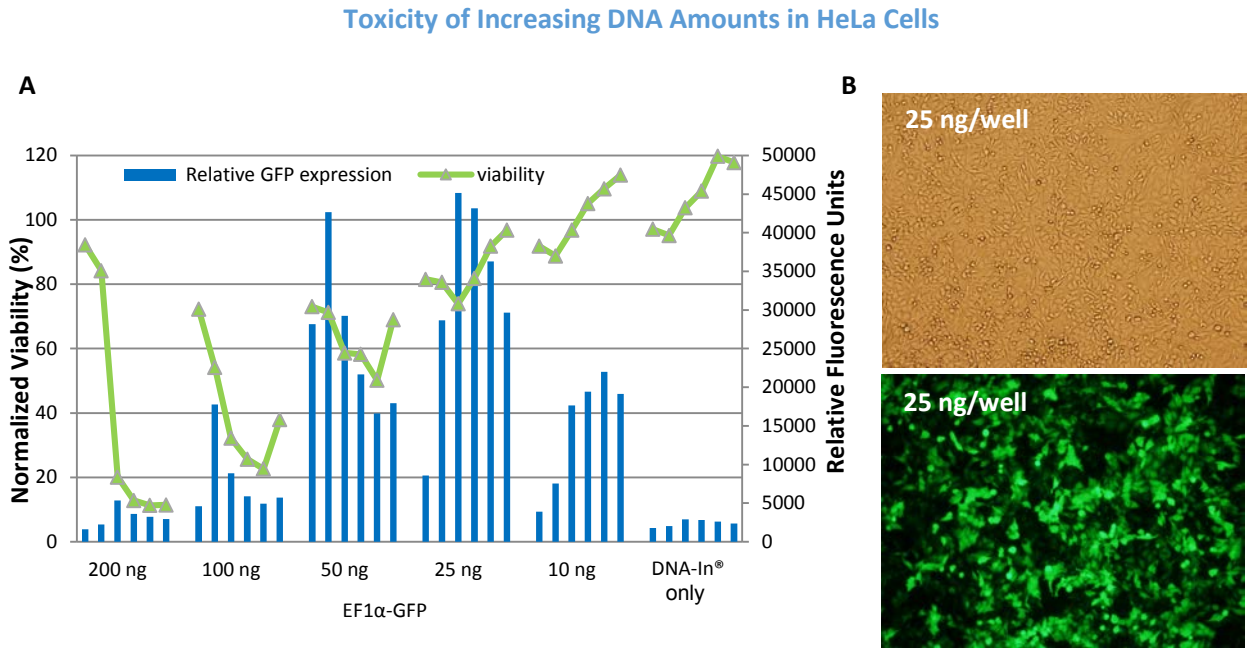

Figure 1. HeLa cells are highly sensitive to DNA transfection-related toxicity. A) HeLa cells transfected with increasing amounts of DNA demonstrated excellent relative fluorescence, indicating successful expression of GFP at lower DNA concentrations (blue bars). However, as DNA amounts increased, GFP expression decreased as cellular toxicity became more evident (green lines). In this graph, the blue bars show GFP expression with increasing concentrations of transfection reagent, from left to right. Green lines represent the viability data from the same well as depicted in the corresponding bar graph. Viability data is normalized to untransfected cells. B) Even at a very low amount of DNA (25 ng/well), almost all HeLa cells exhibited low levels of fluorescence, indicating high transfection efficiency.

Although there was decreased GFP and β-galactosidase expression at lower concentrations, it is important to note that there was very high transfection efficiency at even the lowest DNA concentrations, with the vast majority of cells expressing the foreign DNA (Figure 1B). While the overall expression is lower at lower DNA concentrations, it is still possible to deliver DNA to a high percentage of the cells in the population in what is arguably a more biologically relevant approach.

Human adult fibroblasts (HDFa cells) are a primary cell that also exhibit moderate sensitivity to DNA toxicity following transfection. Similar to HeLa cells, HDFa cells exhibit dose-dependent toxicity following DNA transfection, although the toxicity is less severe than observed with HeLa cells (Figure 2A). Toxicity was not due to the transfection reagent, as indicated by the DNA-In® only data (Figure 2A). Similar to HeLa cells, HDFa cells had high transfection efficiency at even the lowest DNA concentrations, with the vast majority of cells expressing the foreign DNA at even the lowest amounts of DNA (Figure 2B). Similar data was observed for β-galactosidase (data not shown).

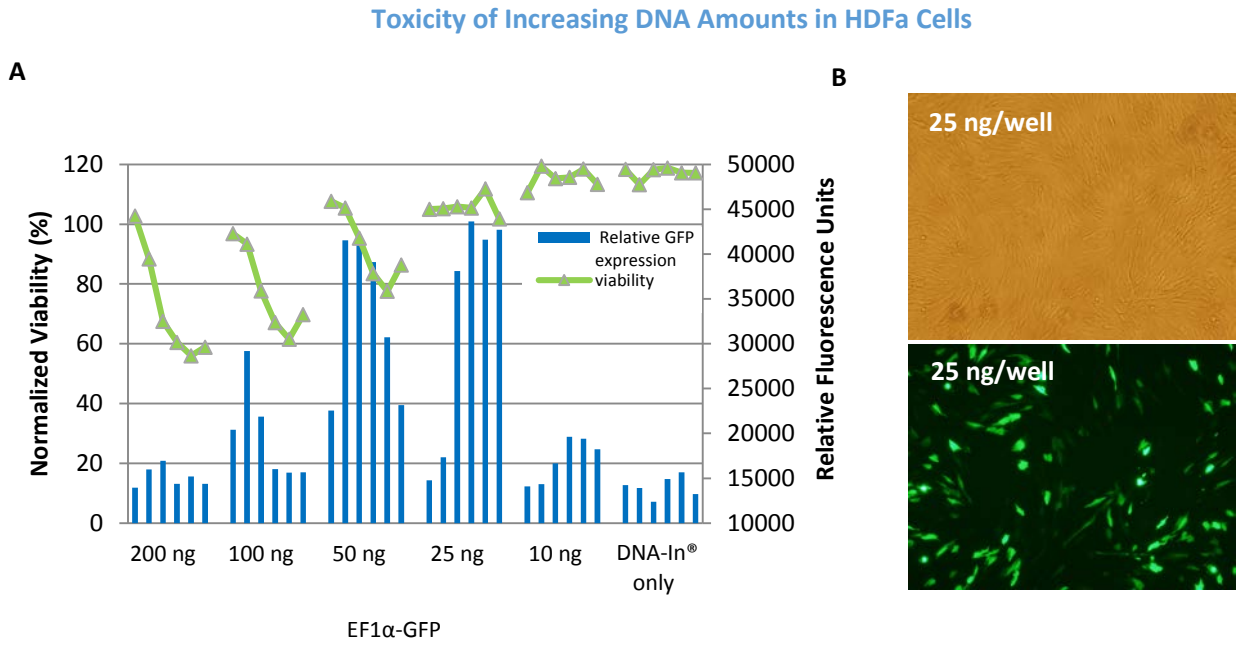

Figure 2. HDFa cells are moderately sensitive to DNA transfection-related toxicity. A) HDFa cells transfected with increasing amounts of DNA demonstrated excellent relative fluorescence, indicating successful expression of GFP at lower DNA concentrations (blue bars). However, as DNA amounts increased, GFP expression decreased as cellular toxicity became more evident (green lines). In this graph, the blue bars show the expression level of GFP with increasing concentrations of transfection reagent, from left to right. Green lines represent the viability data from the same well as depicted in the corresponding bar graph. Viability data is normalized to untransfected cells. B) Even at a low amounts of DNA (25 ng/well), almost all HDFa cells exhibited low levels of fluorescence, indicating high transfection efficiency.

The HeLa and HDFa results indicate that successful transfection occurs at very low DNA volumes, and toxicity is more likely as the DNA concentration increases. However, the very high transfection rates at even low levels of DNA suggest researchers can easily find a balance between sufficient expression and low toxicity.

Relatively insensitive cell lines require less optimization to avoid cellular toxicity

While HeLa and HDFa cells exhibited sensitivity to higher DNA volumes during cationic lipid-based transfection, CHO cells require larger amounts of DNA during transfection to achieve high protein expression, and do not show signs of cellular toxicity at even the highest DNA volumes tested (Figure 3).

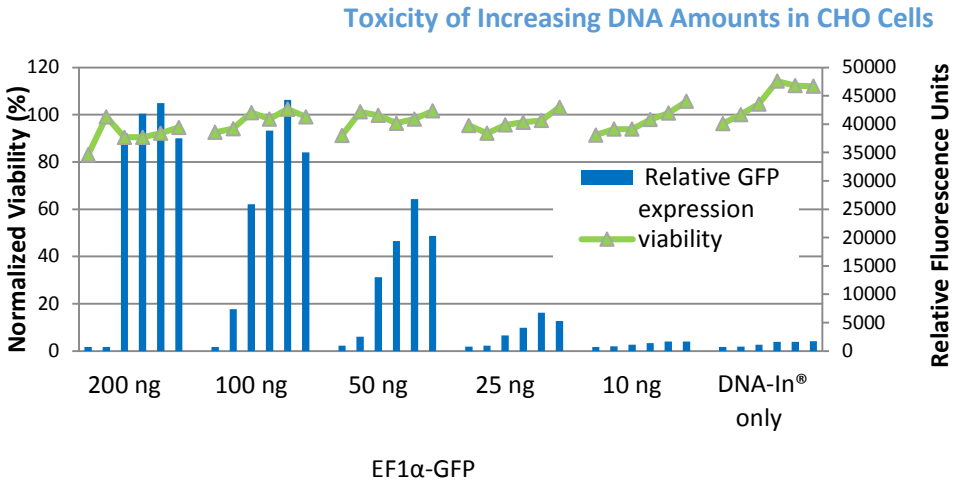

Figure 3. CHO cells are relatively insensitive to DNA transfection-related toxicity. CHO cells transfected with increasing amounts of DNA demonstrated excellent relative fluorescence, indicating successful expression of GFP at lower DNA concentrations (blue bars). Even at the highest DNA concentrations, GFP expression increased in a dose-dependent manner, without any significant cellular toxicity (green lines). In this graph, the blue bars show the expression level of GFP with increasing concentrations of transfection reagent, from left to right. Green lines represent the viability data from the same well as depicted in the corresponding bar graph. Viability data is normalized to untransfected cells.

Summary

Transfection requires a balancing act between introducing foreign DNA into a cell, and not killing the cell in the process. As seen with this data, the toxicity profiles of transfection reactions can be modulated by varying the DNA concentrations used for transfection. In cells where toxicity appears to be an issue at higher DNA concentrations, such as HeLa cells and many primary cells, lowering the DNA concentration to 2.5 µg/ml or lower may be an improved approach over using the more typical 10 µg/ml concentration. For relatively insensitive cells, such as CHO, higher DNA concentrations are not toxic, and may in fact be required to achieve optimal protein expression, providing greater flexibility in planning experiments.

One important caveat to keep in mind is that, depending on the cell type and experimental setup, DNA is not the only potential culprit in causing toxicity during transfection. The transfection reagent itself may be toxic, requiring a transfection reagent only control. Other times, it is the form of DNA that might be toxic. Researchers might consider using CpG island-free DNA or DNA minicircles to minimize toxicity.

Regardless of the chosen approach, researchers can choose to perform optimization experiments in their own lab with a general-purpose transfection reagent such as DNA-In®, or select cell-specific transfection reagents that are pre-optimized, such as DNA-In® CHO and other similar reagents.

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