Using a High Content Screening System to Investigate the Relationship between Transfection Efficiency, Cytotoxicity and Polyethylenimine/pDNA complex localization

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ABSTRACT

A High Content Screening (HCS) system is a powerful method which allows us to gain a lot of information from cell samples. It is possible to correlate the intracellular localization of branch polyethyleneimine/pDNA complexes with transgene expression and to correlate cytotoxicity by using a large data set provided by HCS that can therefore generate unbiased statistically significant data.

In this study, Rhodamine tagged branch polyethyleneimine (Rho-bPEI) and enhanced green fluorescent protein (pEGFP-N1) pDNA were used to prepare the complexes that were subsequently transfected into HEK293 cells. using HCS, 2000 – 8000 cells/well were evaluated. There were 4-5 complexes/cell inside the cytoplasm of GFP(+) cells and less than 1 complex/cell inside the cytoplasm of GFP(-) cells. There were no complexes detected inside the nucleus. Also, cell enumeration recorded by HCS was directly correlated with cytotoxicity assay (MTS assay).

By using HCS, we can rapidly identify and quantify GFP(+) cells, quantify the number of PEI/pDNA complexes within cytoplasm/nuclei and quantify cell numbers. We expect that HCS will allow us to follow internalization pathways of complexes in large populations of cells to provide statistically relevant data in the gene delivery field.

INTRODUCTION

Non-viral gene delivery is a highly promising method for treating various genetic or acquired diseases. The advantages of this approach are low toxicity, ease of preparation and the ability to transport large sized DNA. However, this method also has drawbacks such as low transfection efficiency. In order to overcome this drawback, it may be of benefit to understand the cellular internalization pathway and the localization of the complexes [1, 2]. The aim of this study was to evaluate the performance of a high content screening (HCS, Operetta®, PerkinElmer) system by assessing the capability of HCS to correlate intracellular complex localization with transgene expression and to correlate number of cells with cytotoxicity.
in the HCS system (Figure 1). We found no complexes in nuclei of either GFP(+) or GFP(-) populations. The number of Rho-bPEI/pEGFP-N1 complexes found in the cytoplasm of GFP(+) cells (approximately 4-5 complexes per cell) was higher than the number found in the cytoplasm of GFP(-) cells (less than 1 complex per cell on average) (Figure 2).

Besides determining transfection efficiencies, HCS allows us to quantify cell numbers which can be used as an indicator of cytotoxicity. Nuclei (representing cells) were counted using image analysis software in the HCS system and numbers were compared to the results obtained using a MTS assay. The results from the HCS matched the cytotoxicity results from the MTS assay. Cells that were treated with 1.0 μg pDNA/well showed lower cell numbers as determined by HCS and lower % relative cell viability using the MTS assay than cells that were treated with 0.5 μg/well pDNA.

CONCLUSION
By using HCS, we can rapidly image and quantify the number GFP(+) cells, the number of GFP(-) cells and the number of Rho-bPEI/pEGFP-N1 complexes inside/outside the cytoplasm and nucleus. The number of cells in each well can be correlated to the cytotoxicity as determined by comparison with an MTS assay.

In the future, we plan to create 3D images of complexes inside cells to be able to identify the precise location of complexes. We also plan to look at the relationship of cell roundness and cell area with the cytotoxicity and transfection efficiency.

REFERENCES

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