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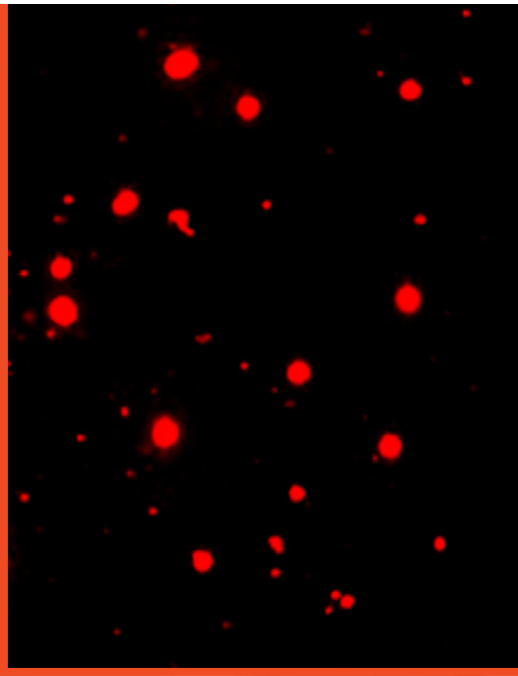

saRNA FROM MAMMALIAN CELL LINES (HEK 293F & VERO) AS A DELIVERY SYSTEM FOR GENE THERAPY PRODUCTS

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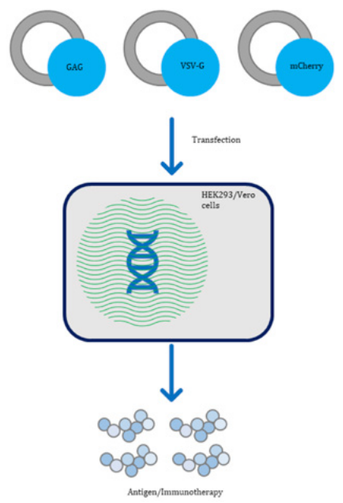
Self-amplifying RNA (saRNA) is a linear and single standard RNA delivery system that can deliver highly immunogenic viral genes and viral peptides to attain rapid and sustained immune response. The saRNA constructs that have been used for gene delivery or vaccine applications have been historically derived from alphaviruses. saRNA's ability to amplify the production of viral antigens in the body is expected to generate a vaccine response at much lower doses compared to traditional mRNA approaches (anti-viral approach). Due to its noble science and manufacturing advantage, a single batch of a low-dose saRNA formulation could offer a rapid, effective, and affordable vaccine solution.

Because it is a large and negatively charged molecule, saRNA cannot be transferred into cells without a delivery system. Several strategies have been adopted to deliver saRNA into cells including LNPs, polymeric nanoparticles and cationic nano emulsions. PEIpro® (Polyplus Transfection, France) is the transfection method of choice. It is effective in high gene transfer efficiency and has been proven to have lower adverse effects and preserve the transfection efficiency.

In this work, transfection performance has been evaluated in mammalian cell lines (HEK 293F and Vero) to produce the saRNA antigens with three various kind of plasmids, including GAG, VSV-G and mCherry (Fig. 1) using PEIpro® transfection reagent. The main advantage of this approach is that the ratios of the expression level of each plasmid, the size of the plasmid, plasmid and diluent ratio and so on can be manipulated, especially during co-transfection of multiple plasmids.

Fig. 1: Transfection flow of HEK 293F and Vero cells



Cells and Culture Conditions

Preliminarily, research cell banks of HEK 293F and Vero cells were prepared from the vials. Cells were grown in a medium appropriate for the cell line and supplemented with serum or growth factors as needed for viability. The optimal growth conditions (temperature: 37°C, CO_2: 5-10%) were facilitated to grow the cells. The cells of 80-90% confluency were stored at below -150°C vapor phase of liquid nitrogen. BHK-21 adherent cells, which were used for the analysis of transfected cells, were grown in MEM media (added 10% FBS) with 80-90% confluency.

Transfection Procedure

Transfection efficiency of plasmids was optimized based on the varying amounts of plasmid DNA, transfection reagent concentrations and various diluents (FS293 media, serum free DMEM, PBS and HEPES). The transfection procedure is illustrated in Fig. 2.

Fig. 2: Transfection procedure of HEK 293 and Vero cells.

		Timeline	Steps
Day 0	1		Seed cells to be 50-80% confluent at transfection
Day 1	2		DNA and PEIpro® dilution by HEPES buffer
	3		DNA and PEIpro® dilutions incubation
	4		Add diluted DNA to diluted PEIpro® and incubation (15 min.)
	5		Add DNA- PEIpro® complex to cells (HEK 293 or Vero) and mix
	6		Incubate the DNA-PEIpro® complex and cells for 1.5 h at 37°C with 8% CO_2
	7		Centrifugation (2000 rpm, 2 min.) and add to 6 well plate
	8		Add 3% of Xell feed
Day 2-3	9		Incubation (24-48 h)
	10		Visualize/Analyze transfected cells

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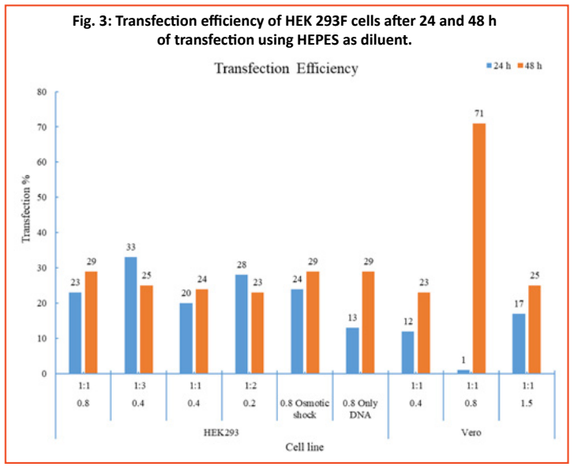
Analysis of Transfected Cells

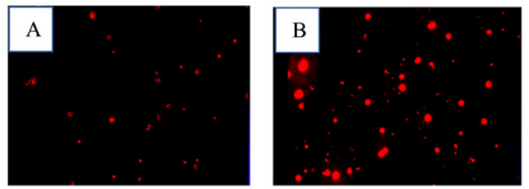
The viability, total cell density (TCD), percentage of TxRed cells (data not shown) and percentage of transfection were analyzed after transfection of cells. The analysis was carried out at 24 and 48 hours post transfection. The combination of 0.8 µg DNA, 1:1 DNA: PEIpro® and HEPES diluent has achieved a better transfection efficiency in both the HEK 293F (23%) and Vero (71%) cell lines for 24 and 48 h, respectively (Fig. 3).

Fig. 3: Transfection efficiency of HEK 293F cells after 24 and 48 h of transfection using HEPES as diluent.



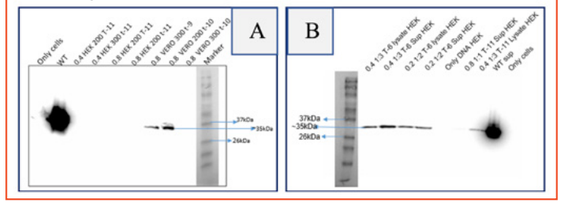
HEK 293 and Vero cells transiently transfected with DNA for mCherry red fluorescent protein (RFP) expression. The images of HEK 293 (Fig. 4A) and Vero cells (Fig. 4B) revealed that the transfection of plasmids was efficiently befallen into the cells.

Fig. 4: HEK 293 and Vero transfection with DNA concentration and DNA: PEIpro® ratio and HEPES diluent.



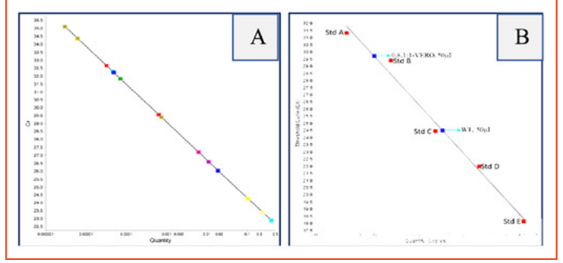
The confirmation of transduction was evaluated by Western blot and RT-PCR techniques. Briefly, western blot analysis was performed with the inoculation of supernatants from transfected HEK 293F and Vero cells into BHK 21 cells. The membrane was developed with ECL substrate and exposed for the detection of saRNA antigens. From the results, it can be seen that the Vero cells have presented a characteristic band at 35 kDa, which resembles the successful expression of saRNA antigen (Fig. 5A). Surprisingly, for none of the HEK 293F cells tested, a substantial particle expression for saRNA could be observed (Fig. 5A). As a result, lysates from HEK 293F cells were blotted to know the expression level and interestingly, bands were appearing at 35 kDa (Fig. 5B), which indicate that saRNA antigens were not secreted into the medium.

Fig. 5: Western Blot results of BHK 21 cells infected with supernatant and lysates collected from transfected HEK 293F and Vero cell lines.



RT-PCR has also been carried out to confirm the transduction of plasmids into HEK 293F and Vero cells. The supernatants from the HEK 293F and Vero cells were transducted into BHK 21 adherent cells, amplified the DNA and genes, and plotted against the standard DNA concentrations. The threshold cycles (Ct) of supernatant from Vero cells were found to be in-line with the standard curve, which confirm the transfection of plasmids into Vero cells (Fig. 6B). In contrast, supernatant from HEK 293F cells has not reported any amplification, therefore, RT-PCR with two different probes (MC and NSP) has been conducted with lysate samples. The results suggest that the Ct values of lysate samples were within the linearity (Fig. 6A) of standard quantity obtained and in accordance with the western blot result.

Fig. 6: RT-PCR analysis



Overview Summary

Transfection of three plasmids (GAG, VSV-G, and mCherry) was successfully accomplished into both the HEK 293F and Vero cell lines by optimizing the transfection protocol to produce saRNA antigens. Promising results were achieved from the optimized transfection protocol in terms of efficiency and expression and the same was confirmed by the analytical techniques. In conclusion, attractive expression system was developed by optimizing transfection protocol, which leads to produce a wide range of gene therapy products replacing mCherry with our gene of interest.

References

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PEIpro®, DNA transfection kit for virus production from Polyplus Transfection.

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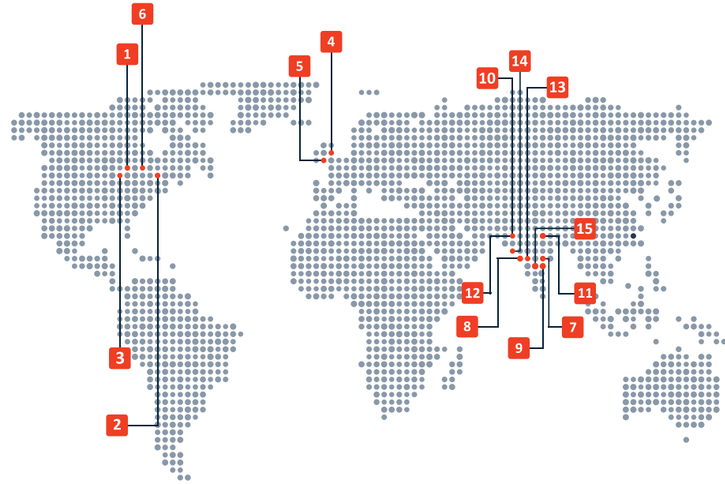

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