

Transfecting myocytes with Viromers®

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1. Introduction

Myocytes are long, tubular cells that develop from myoblasts to form muscles and include a number of specialised cell types, including cardiac, skeletal and smooth muscle cells, each with specific properties. Given that myocytes are important for essential body processes such as heart contraction and can malfunction to cause a wide range of diseases, many researchers are interested in studying how they work.

Genomic approaches such as gene knock down or overexpression enable researchers to rely upon one key thing: transfection. Unfortunately, myocytes are very difficult to transfect. This fact has held researchers back from quickly, efficiently and productively exploring how they function under normal physiological circumstances.

It also limits us from understanding their role in the development of a large number of important cardiovascular and

cytoskeletal diseases, reducing our ability to develop new treatments

Lipocalyx has therefore developed Viromers® – a series of novel chemical polymers that deliver DNA and RNA into cells with high efficiency, without impacting on cell viability. By mimicking the uptake of the influenza virus, they can safely enter cells via the endosomal escape pathway.

2. Successfully transfecting myocytes

Viromers vastly improve the transfection efficiency of siRNAs, plasmids, mRNAs and other oligonucleotides safely into 'hard-to-transfect' cells such as myocytes. Viromers are synthetic polymers that take advantage of the molecular mechanism that the influenza virus uses to enter cells, known as 'active escape via the endocytic pathway' (Figure 1). This allows researchers to generate high quality, reproducible results and understand the biology of myocytes in more detail than ever before.

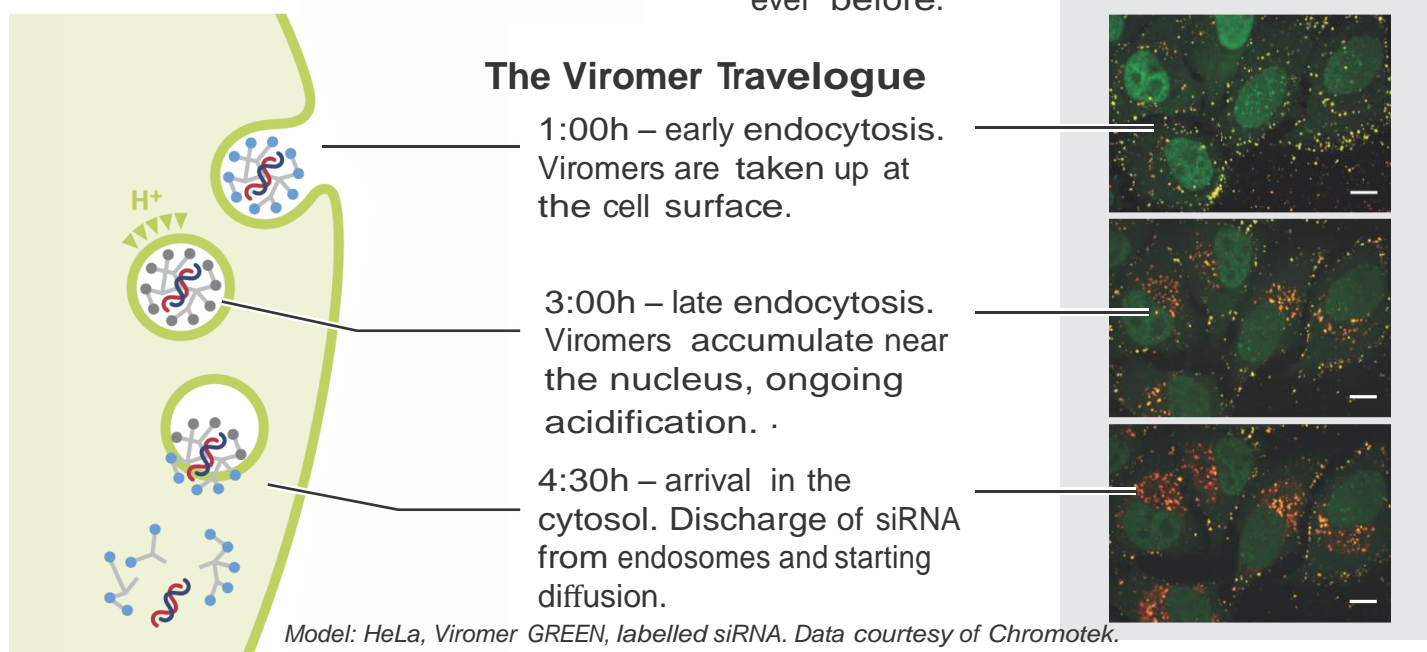


Figure 1. The active escape via the endocytic pathway.

3. Viromers® in action

3.1 Case study: gene overexpression studies in C2C12 cells to understand the effects of obesity on muscle cell metabolism

As a mouse myoblast cell line capable of differentiation, C2C12 cells are an extremely useful tool in the study of how muscles form. As such, functional genomic studies investigating the pathways controlling C2C12 differentiation provide key insights into myogenesis, metabolism and muscle biology.

Dr C.L. Tse at the University of Oklahoma is currently studying how obesity can cause metabolic changes in muscle. The team has been working to overexpress a number of transcription factors in C2C12 cells in order to understand their effects on the gene expression profiles of muscle cells. In order to overexpress the transcription factors of interest, Viromers have been used to transfect plasmids carrying the relevant genetic material into the C2C12 cells (using the protocol outlined below).

- First, the cells, DNA construct and Viromer Mix were prepared
 - C2C12 cells were seeded in antibiotic-free medium using a 6-well format and left overnight to achieve 80% confluence
 - A solution containing the DNA expression construct (GFP) was created by diluting DNA to 18 ng/μL
 - Viromer Mix was created by mixing 2.5 μL of Viromer stock with 60 μL of buffer E

- Next, the DNA construct was combined with the Viromer Mix and incubated with the cells to trigger uptake
 - 60 μL of Viromer Mix were mixed with 340 μL DNA solution
 - The mixture was left at room temperature for 15 minutes so that the DNA construct would be complexed with the Viromers
 - 200 μL of the complexed mixture was added to the C2C12 cells (using antibiotic-free medium)
 - The complexed mixture was left to incubate with the cells for 24–48 hours (until the cells reached 90–100% confluence)
 - The cell fusion medium (dMEM+2%HS+PS) was then changed, followed by 4 more changes (once per day, over 4 days)
- Finally, GFP expression from the construct was confirmed
 - GFP expression was assessed using microscopy to ascertain the uptake of the GFP construct.

Although these experiments are still underway within the lab, this initial validation study has allowed the team to make real progress. It has also given them confidence that they can now transfect C2C12 with the efficiency required to obtain insightful data when moving on to more biologically interesting constructs.

“We have tried the Viromer on undifferentiated C2C12. The myotube remains being transfected and it works great.”

Dr C.L. Tse, University of Oklahoma, Health Science Center, Department of Physiology

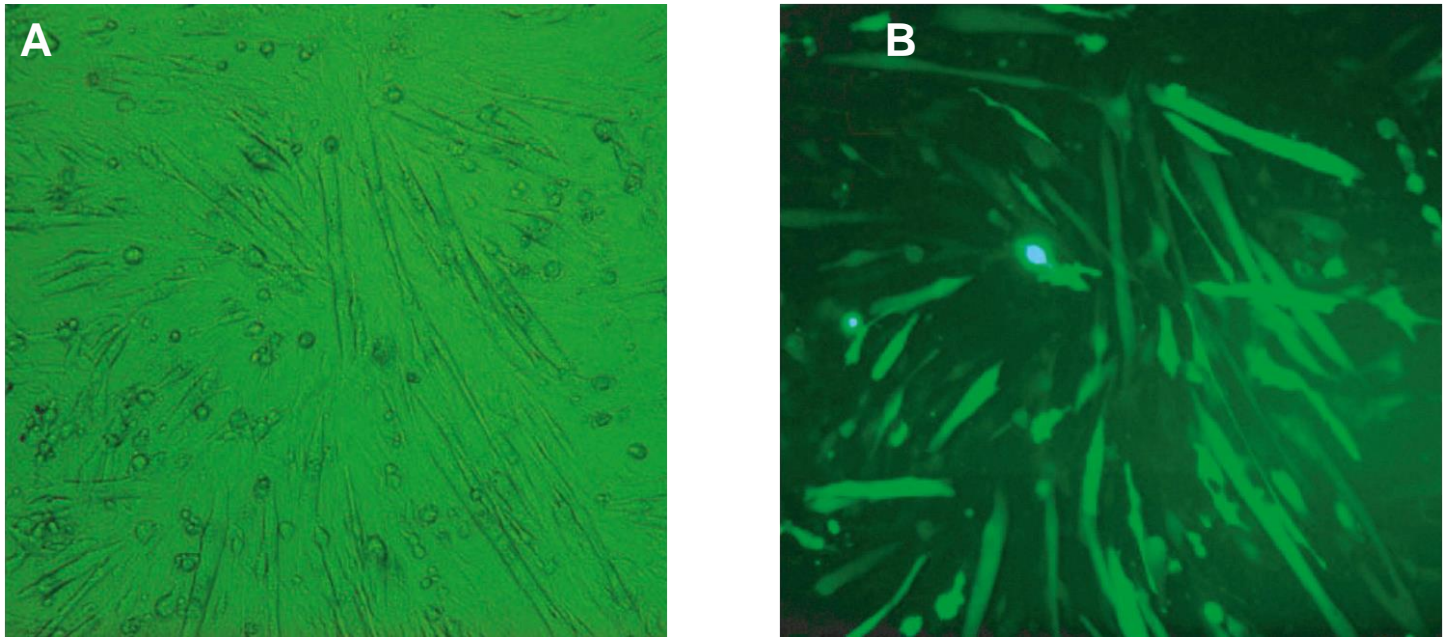


Figure 2. GFP expression in C2C12 cells which have been successfully transfected using Viromer® RED. (A) Phase contrast, (B) GFP-1.

3.2 Case study: an investigation into the pathophysiology of primary cardiomyocytes to determine the effects of hypoxia on ion channel function

Cardiac muscle is the autonomously-contracting, striated muscle found exclusively within the heart. Primary cells derived from cardiac muscles are especially difficult to transfect and are often referred to as ‘super-resistant’. Dr A. Castellano and his team at the Institute of Biomedicine, Seville, are interested in the role of ion channels in the pathophysiology of cardiac muscle, which is essential for muscle contraction, and are analysing the effects of hypoxia on cardiac ion channel expression and function.

Using plasmids with different promoters linked to the expression of a GFP reporter, they hope to assess which transcription factors might be involved in mediating this response. Primary neonatal rat ventricular cardiomyocytes were transfected with a GFP plasmid construct, and uptake of the construct assessed using microscopy. The team used the following protocol:

- Primary neonatal rat ventricular cardiomyocytes were collected
- Cells were plated in a 24-well culture plate at 10^5 cells/well
- The cells were cultured for 24–48 hours and transfected with $0.5 \mu\text{g}$ of a plasmid expressing GFP
- The standard protocol and lowest concentration of Viromer YELLOW were used
- The cardiomyocytes were incubated with Viromer YELLOW for 4 hours, after which the medium was changed
- GFP expression from the construct was confirmed using microscopy to ascertain the uptake of the GFP construct (as shown in Figure 3)

In this study, Viromer YELLOW provided 50% transfection efficiency with very low toxicity.

The team’s research is still ongoing, however if they obtain positive results in the reporter gene studies, the next step will be to confirm the results using chromatin immunoprecipitation (ChIP) assays.

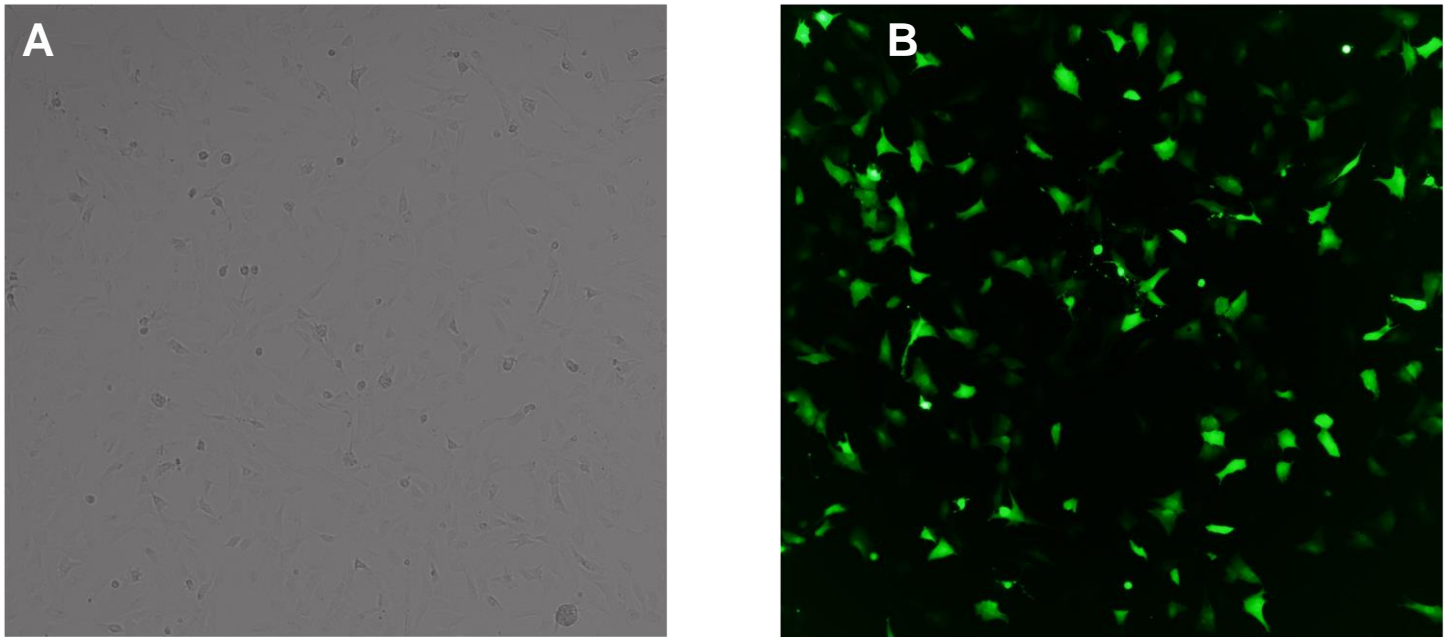
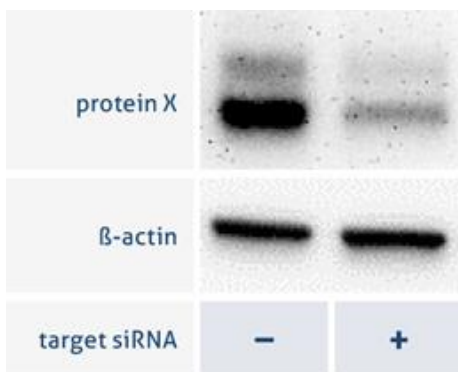


Figure 3. Primary neonatal rat ventricular cardiomyocytes successfully transfected with a GFP-encoding plasmid using Viromer® YELLOW. (A) Light microscope image, which shows good cell viability and (B) GFP-overexpression.

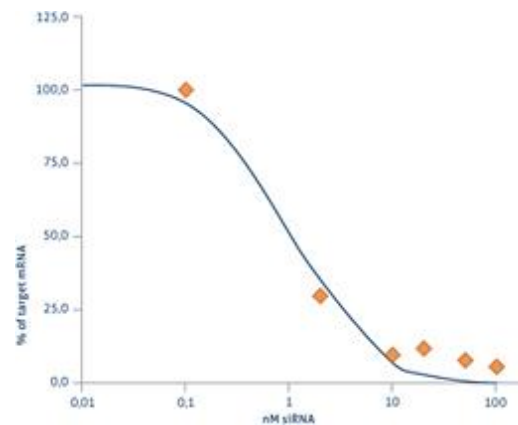
“Viromer YELLOW works much better than the other reagents we have used before to transfect neonatal rat cardiac myocytes. We obtained 50% efficiency with low toxicity.”

Dr A. Castellano, Institute of Biomedicine of Seville.

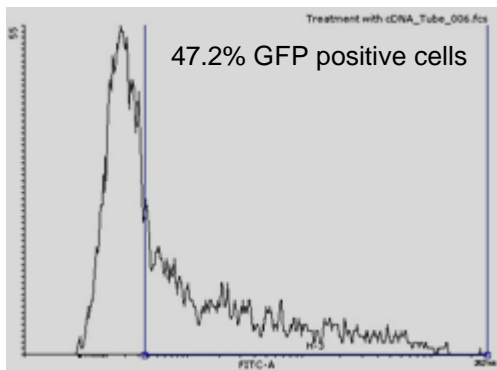
3.3 More data



C2C12 cells, siRNA transfection, Viromer Blue



Primary Human myoblasts
siRNA transfection, Viromer Yellow



H9C2 cells, GFP plasmid transfection
Viromer Red

Conclusion

Myocytes have traditionally been difficult to transfect, which has held researchers back from carrying out biologically interesting functional studies. Viromers are a new alternative that offer a significant improvement in transfection efficiency, while also ensuring cell viability. By using a novel active endosome escape, they reduce background noise, forming stable complexes with siRNA/miRNA, plasmid DNA and mRNA to provide reliable and reproducible results.

Viromers are also compatible with serum and lipid-free antibiotics, meaning there is no need to change to a reduction media during the experiment. In addition, Viromers do not interact with the metabolism of cells, producing high quality functional data that can be trusted.

To learn more about how Viromers can enable functional studies with myocytes, please visit www.lipocalyx.de/myocyte-transfection.



Cat# [TT100302](#)



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