Microfluidics™

Application Note

Microfluidizer® Technology in Cell Lysing for Gene Therapy

Virus used in tests and Microfluidizer Homogenizer

INTRODUCTION

Gene Therapy (GT) is a way to treat disease at the genetic level. If a gene or nucleotide can be identified that is responsible for a disease, the theory is replacing that gene will cure the disorder. GT has been proposed to treat congenital blindness, hemophilia, Parkinson's, Multiple Myeloma and many other diseases. To date several GT products have been approved and commercially available globally.

There are several ways GT can be used: a mutated gene can be replaced, a mutated and dysfunctional gene can be knocked out, or a new gene can be introduced to help the body fight off the disease. To deliver those therapeutic genes, the most commonly used carriers are viral vectors due to several advantages associated with their properties. Viruses can infect cell efficiently, be able to target a wide range of host cells and achieve high levels of transfection, and some viral vectors can even transduce non-dividing cells and are also non-pathogenic. Four main classes of viral vectors used in preclinical and clinical applications are adenoviruses, adeno-associated viruses (AAVs), retroviruses, and lentiviruses. Adenoviruses and AAVs are sometimes preferred over the other two classes since they do not integrate themselves into the genome.

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WHEN CELL LYSING IS REQUIRED

Virus production involves multiple upstream production processes and downstream purification steps. Developing and optimizing scalable viral vector production, harvest and purification processes is critical and can be challenging. Virus preparation methods for bench top scale usually include procedures that are difficult to scale-up, time and labor consuming and present residual contamination issues. Vectors such as AAVs are commonly grown in mammalian cells (HEK) cells or insect cells and therefore, cell rupture is required to release and harvest those vectors. The conventional method for lysing cells within the manufacturing environment has been multiple rounds of freeze-thaw cycles. However, the potential contamination issues associated with the freeze-thaw process make it less than ideal way for large scale production. Furthermore, the process is both time and labor intensive.

THE MICROFLUIDIZER TECHNOLOGY

The Microfluidizer technology is the most ideal alternative cell lysing method for harvesting vectors. The unique design of the Microfluidizer technology ensures that fluid pressure is converted to highly controlled shear forces more efficiently and consistently (Figure 1) than is readily achievable using competing technologies. The key to the Microfluidizer technologies remarkable efficiency is the design of our exclusive fixed-geometry interaction chamber. As cells are forced through the interaction chamber under desired constant pressure and controlled temperatures, the exact right amount of shear force is applied uniformly to rupture the cell membranes without damaging the viruses. Plus, the Microfluidizer technology is seamlessly scalable from lab to production and a range of cGMP ready processors are available.

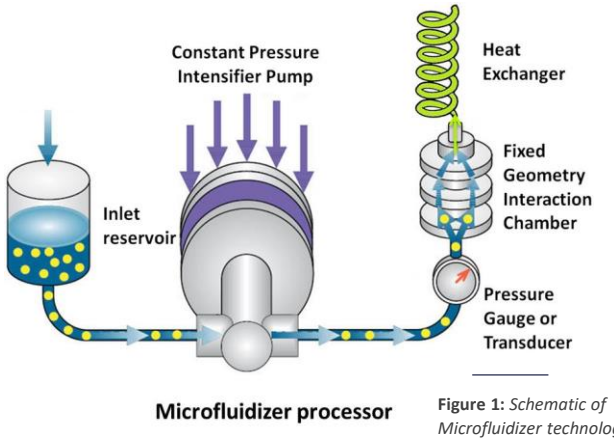

Figure 1: *Schematic of Microfluidizer technology*

A CASE STUDY

A case study is presented here to compare the virus recovery efficiency between freeze-thaw method and the Microfluidizer technology. In this study, AAV vectors were grown inside HEK293 cells and resuspended in Tris buffer before lysing using both methods. In freeze-thaw method, 5 repeated cycles of freezing cell suspension in dry ice/isopropanol bath and then thawing it at 37°C were performed to rupture the cells. In the Microfluidizer homogenization method, cell suspension was processed once through the Microfluidizer processor equipped with a H30Z (200 µm) interaction chamber at 4,000psi (275 bars).

In both cases, the lysate was centrifuged for 30 minutes to remove debris before analyzed by droplet digital polymerase chain reaction (ddPCR) to quantify AAV titers. Microscopic images of cells before and after the Microfluidizer homogenization step showed in Figure 2 clearly indicates that one pass was able to rupture almost all the cells.

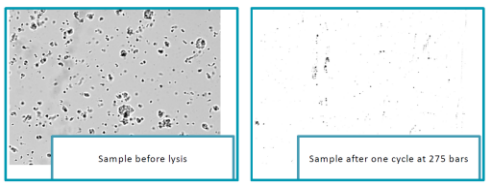

Figure 2: *Microscopic images of cells before and after the Microfluidizer homogenization step*

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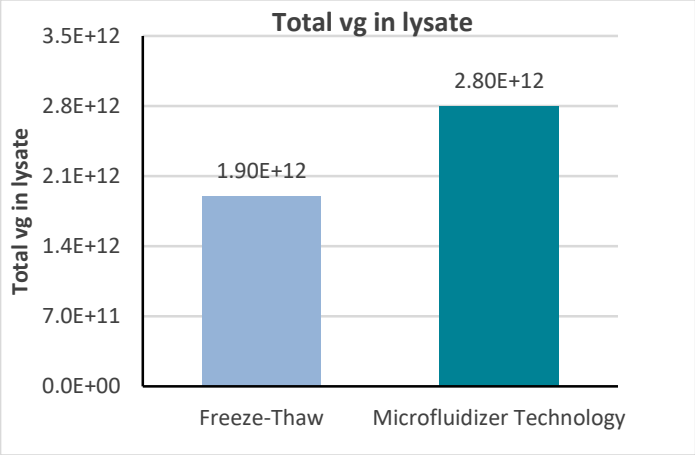

Figure 3: *Amount of AAV recovered after cell lysing with freeze-thaw method & Microfluidizer technology*

Figure 3 shows that under such high efficiency, the amount of AAV harvested using the Microfluidizer technology is significantly, about 50%, higher than that obtained through freeze-thaw method. Furthermore, the freeze-thaw method took 5 hours to complete while the entire Microfluidizer process was done in less than an hour including all preparation and sanitizing steps.

Virus production involves multiple upstream production processes and downstream purification steps. Developing and optimizing scalable viral vector production, harvest and purification processes is critical and can be challenging. Virus preparation methods for bench top scale usually include procedures that are difficult to scale-up, time and labor consuming and present residual contamination issues. Vectors such as AAVs are commonly grown in mammalian cells (HEK) cells or insect cells and therefore, cell rupture is required to release and harvest those vectors. The conventional method for lysing cells within the manufacturing environment has been multiple rounds of freeze-thaw cycles.

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THE KEY BENEFITS OF MICROFLUIDIZER TECHNOLOGY

In summary, Microfluidizer technology provides a number of benefits in viral vector production:

High yield – Effectively ruptures cells to release viruses without damaging vectors. Process through Microfluidizer can also potentially avoid virus agglomeration and binding to membranes, which drastically reduce vector yield, thus achieve higher yield compare to other methods.

Scalability – Scale up from lab to production linearly with guaranteed results.

cGMP ready – Biopharmaceutical models are engineered with full cGMP compliance.

Easier for downstream processes – Break cells gently yet efficiently, resulting in large cell wall fragments which are easier to separate from the much smaller viral vectors. The process can also be set to shear the cellular DNA and reduce viscosity. Both make the downstream clarification and filtration processes much easier.

No chemical/enzyme contamination – Can achieve media-free, negligible-wear processing. No need for chemical lysis method, which means no extra detergent removal step and simplified downstream processes.

No costly chemicals – Do not need or can significantly reduce the amount of costly nuclease such as Benzonase.

Manufactured by Microfluidics, distributed in the UK and Ireland by **analytik**.