Microfluidics™

Application Note

Cell Disruption with Microfluidizer® Technology

Ths scalable M-110P

INTRODUCTION

The next generation of drug development utilizes intracellular contents such as proteins, organelles, DNA/RNA, enzymes and Adeno-Associated Virus (AAVs) Vectors that are found and/or grown inside cells.

For cells that do not secrete these intracellular contents, it is vital to lyse the cell to liberate the contents. Finding the best suited cell disruption method among the many available ones, however, isn't always that easy.

It is important to achieve high rupture rates, but at the same time not to denature intracellular components by unnecessary elevation of temperature or excessive shear rates which will adversely affect yield rates and protein activities.

The method applied should be easy to use, reliable, and require little maintenance and be easy to clean. It should also be able to deliver repeatable and, ideally, scalable results. Flexibility with regards to volumes and cell types to be lysed is also desirable.

This application notes compares some of the most commonly used cell disruption techniques with regards to these criteria and highlights feedback from notable researchers who have had the opportunity to evaluate a variety of different methodologies.

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

Cell Disruption with Microfluidizer® Technology

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	Microfluidizer	Homogenizer	Bead Mill	French Press	Sonication
Principle	Fixed-geometry interaction chamber and constant pressure pumping system. Uniform and highly precisely controlled shear rates.	Variable valve geometry in combination with less constant pressure profile. Less controlled energy input/ less uniformity.	Cylindrical rotating shell partly filled with beads that fall onto the material to be ground. Forces applied are impact and attrition.	Pressurization and decompression depends on a manually operated valve. Speed of human user's valve turn determines actual applied shear.	Uses cavitation to generate shear – typically much lower than high pressure methods. Increasing shear results in higher processing temperatures.
Continuous	Yes	Yes	No	No	No
Scalable	Yes	Limited	Yes	No	Limited
Optimal Temp Control	Yes *(cooling coils or heat exchangers)*	Yes *(cooling coils or heat exchangers)*	No	No	No
Contamination Free	Yes	Uncertain	No		
Minimum Volume	1ml	10 ml	1 ml	1 ml	< 1ml
Constant Shear Rate	Yes	No	No	No	No

COMPARISON OF METHODS

Compared to alternative cell disruption methods, the Microfluidizer® Processor offers several advantages to benefit from:

- ease of use
- easy to clean
- reliable
- quick processing time
- lowest required pressures
- efficient cooling for high yields & activity levels
- no contamination
- repeatable and scalable
- usable for variable cell suspension volumes and a large variety of cell types
- customers report lower viscosity and turbidity after processing which simplifies down stream processing

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CELL RUPTURE SUCCESS STORIES

Hear what our customers have to say about Microfluidic's technology for Cell Rupture:

Prof. Thomas Schwartz, Massachussetts Institute of Technology (MIT) (USA)

"I highly value the Microfluidizer® Machine for cell disruption. It's very reliable, processing hundreds of liters of our diverse cell suspensions with very few problems.

My colleagues and I have used two different brands of competitive equipment, but now have switched exclusively to Microfluidics. The difference is night and day. Yields of usually difficult, otherwise poorly soluble proteins are much higher after disruption with the Microfluidizer® processor.

Maintenance requirements are also much lower now. With the competitive systems, maintenance was a constant problem and required several service visits per year. Overall, I am very happy that I switched to the LM20 Microfluidizer® processor."

Dr. Sven Hennig Associate Professor Structure Chemical Biology- Head of X-ray Crystallography, Vrije Universiteit Amsterdam, Netherlands

"We used to sonify our bacterial samples. But only with the Microfluidizer it is possible for us to lyse sample sizes from a few ml up to a few hundred ml.

Additionally, our samples don't heat up that much anymore during cell lysis.

For us it is the most efficient and easy way to rupture cells in a reproducible manner, so cell lysis is no trouble anymore. We are happy to have it."

Johannes Raff, Ph.D., at the Institute of Radiochemistry at Forschungszentrum Rossendorf, Germany

They used a French pressure cell, ultrasonification, a glass bead mixer mill, and homogenizers to disrupt various bacterial cells until a colleague told him of a high-shear fluid processor that was much more efficient and easy to use.

Raff tested the Microfluidizer® processor and stated: *"We can process 200 ml of 1:1 solution continuously in five to ten minutes versus constantly refilling the chambers of other types of equipment over a two- to three-hour time span.*

The Microfluidizer® disrupts 99% of the bacteria cells in two to three passes versus the five to ten passes other cell rupturing equipment requires."

James Wright, Protein Scientist - Abcam Plc, UK

"The LM20 Microfluidizer processor offers superior lysis over traditional methods of cell disruption with an efficient system to reduce internal blockages.

The scalability of the design means that the forces experienced by cells will not change if the sample volume increases. I can, therefore, have confidence that the sample quality, yield and processing times won't be impacted by scaling up my lab processes.

Digital controls allow the system pressure to be set more easily and accurately compared to alternative manual processors, reducing the potential for errors and increasing reproducibility.

The in-depth user training, and easy operation and maintenance procedures have been extremely beneficial. I feel confident to troubleshoot and perform preventative maintenance without having to call the support teams - it doesn't feel like a 'black box' machine.

I would recommend the LM20 Microfluidizer as an essential tool for cell disruption."

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CELL RUPTURE SUCCESS STORIES

Hear what our customers have to say about Microfluidic's technology for Cell Rupture:

Dr Petra Ungerer, Lab Manager, School of Biological & Chemical Sciences at Queen Mary University London, UK

"We identified Microfluidizer technology as a replacement for a high-pressure homogeniser from another manufacturer, which had been unreliable and failed to achieve sufficient lysis of our tough yeast cells even after very many passes.

We selected the LM20 (fig 1) Microfluidizer not only because it efficiently lysed a broad range of cell types but also was incredibly simple and easy to use which is very important as many different people need to use the machine regularly.

We also much appreciated the knowledgeable technical and applications support provided by the local representative which has enabled us to fully exploit the considerable capabilities of the LM20 Microfluidizer."

Figure 1: *The LM20 Microfulidizer technology*

Dr. Julien Hiblot at Max-Planck-Institute for medical research in Heidelberg, Germany

"The Microfluidizer® allows us to reach high performance in producing active protein for biochemical and structural biology studies.

We use the Microfluidizer® for bacterial cell lysis, it is the method of choice when it comes to difficult proteins or large culture volume.

The equipment is easy to use, easy to maintain and offers reliable results."

Dr Michael Chen, Nuclera co-founder & CCO

Nuclera is a synthetic biology company developing a next-generation DNA synthesis and automation platform based on engineered terminal deoxynucleotidyl transferases (TdT) to enable the production of gene and genome libraries.

The company already boasts 3 patents to their name and has a long-term vision to create a DNA synthesis platform capable of manufacturing genomes on-demand.

The company chose the Microfluidizer LM20 (fig 1) for cell disruption, or the releasing of biological molecules such as proteins and enzymes, from inside the cell.

"I had been using a competitor system to perform 100-500 ml E.coli lysis for more than a year during my PhD. When I switched over to a Microfluidizer lysis machine, the difference was night and day. Breakage efficiency was higher and instrument blockage from particulates in the lysis buffer was far less problematic. A Microfluidizer machine made processing E.coli lysates much more convenient, so I could worry about enzyme function rather than production"