Microfluidics™

Application Note

Microfluidizer® Technology for creating RNA vaccine delivery systems

INTRODUCTION

Next generation RNA vaccines have been the focus of vaccine research since the early 2000s.

They are particularly useful in pandemic responses because the production of the antigen is not dependent on complicated production methods.

However, RNA by itself is not viable as a vaccine so a delivery system is usually required. To overcome this limitation, various viral vectors and non-viral nanoparticles have been explored as carriers.

Lipid-based nanoformulations such as lipid nanoparticles (LNPs), cationic nanoemulsions (CNEs), nanostructured lipid carriers (NLCs), are the most advanced systems and have shown great promise.

Two approaches are commonly adopted when using nanoparticles to deliver RNA molecules - the in-situ encapsulation approach or post adsorption approach. The latter is essentially a two-step approach which was explored in a published scientific paper:

A Nanostructured Lipid Carrier for Delivery of a Replicating Viral RNA Provides Single, Low-Dose Protection against Zika[1]

This application note summarizes the article and shows why the approach is suitable for large scale manufacturing & responding to pandemic situations.

Microfluidizer® Technology creating RNA vaccine delivery systems

RNA VACCINES

Traditional virus vaccines are typically grown in chicken eggs and second generation subunit protein vaccines are typically grown in bacterial cells. Both these tissue based production methods require a lot of time for optimization and validation of the production and purification processes.

Nucleic acid based vaccines, on the other hand, can be developed and produced much more quickly. However, significant challenges exist in delivering these molecules, primarily due to their instability under physiological conditions, so a delivery system is required when formulating the RNA based vaccines.

DELIVERY OF RNA WITH NANOSTRUCTURED LIPID CARRIERS (NLCs)

Various lipid-based nanoformulations, including LNPs, CNEs, and NLCs, have been widely used to deliver RNAs either via direct encapsulation or post adsorption.

The encapsulation approach requires the LNPs to be manufactured in the same vial. Drawbacks of this include challenges with formulation stability and scalability.

The adsorption approach allows the delivery system to be produced on a mass scale and stored in preparation for a pandemic outbreak. When the pathogen for the outbreak is established, RNA antigen can be developed and produced quickly. The RNA is then added to the NLC formulation and binds to the surface of the particles. This two-step approach is a more versatile alternative to LNPs and ideal for responding quickly to a pandemic situation.

The article proposes the use of the latter route. The authors were able to develop a novel platform – Nanostructured Lipid Carrier (NLC) as the delivery system for a Zika vaccine candidate.

NLC FORMULATION

The NLCs developed in the research for this paper are a hybrid formulation comprising the following components:

- Squalene oil built part of the nanoparticle's core. Microfluidizer® technology has been used for decades to create squalene-based emulsions which are used widely as vaccine adjuvants.
- Dynasan 114, a solid phase lipid, was used in some formulations as it has been shown to help increase circulation times inside the body.
- Two polymeric surfactants Span 60 and Tween 80 were used to stabilize the nanoparticles and enhance bioavailability.
- Finally a cationic phospholipid Dioleoyl-3-trimethylammonium propane (DOTAP) was added to allow for the binding of the positively charged RNA.

The nature of this hybrid core formulation forms a semi-crystalline matrix that provides excellent nanoparticle stability while maintaining the ease of manufacturability.

Microfluidizer® Technology creating RNA vaccine delivery systems

NLC FABRICATION

The desired nanoparticle production method should not only be able to produce NLCs with targeted properties, e.g. particle sizes, long term colloidal stability, and be filter sterilizable, etc., but it also needs to provide flexibility in terms of creating NLCs with varying physicochemical properties during development stages as well as suitable for future process scalability.

Microfluidizer® technology can meet all of these requirements, generating small and uniform nanoparticles via the uniform high shear rates produced by the combination of fixed geometry Interaction Chamber™ microchannels and constant pressure processing up to 2,000 bar (30,000 psi). The technology is linearly scalable up to thousands of liter batches and capable for cGMP manufacturing.

In this study, all NLC formulations were manufactured by processing 5 passes at 30,000 psi through an M110P Microfluidizer® processor (figure 1).

Figure 1 – M110P Microfluidizer® processor

Reference *[1] J. H. Erasmus, et al., Mol. Ther., 2018, 26(10).* To link to the original paper click here.

RESULTS

Unimodal NLCs with an average size of less than 100 nm were achieved in all the samples, which were sterile filtered.

Self-replicating RNA-encoding Zika virus antigens were selected. The RNA was added after processing to achieve the final NLCs as pictured in figure 2.

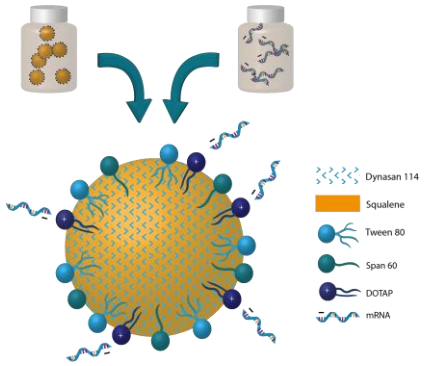

Figure 2– Schematic representation of RNA surface adsorbed to NLC nanoparticle post-admixture

Once the NLC manufacturing process had been successfully developed, the composition was optimized for loading capacity, safety and immunogenicity. 100% RNA-binding at concentrations up to 10mg/mL was achieved with total degradation protection from RNase.

The article concluded that a single dose with as low as 10 ng of RNA can develop sufficient immunity to protect against an otherwise lethal Zika Virus challenge.

HOW MIGHT THIS HELP WITH COVID-19?

Although the research carried out in this article was aiming at the Zika virus, the method and technique described in it paved a potential path for delivering RNA for other vaccines or treatments, including those fighting against the COVID-19 pandemic.