Pathbreaking method leads to optimised mRNA production

The COVID-19 pandemic placed mRNA at the centre of biopharmaceutical research, as mRNA is now being developed for cancer therapy, protein replacement therapy, and infectious diseases. That is why, worldwide, the need to produce mRNA on a large scale has increased dramatically. The currently used method is quite costly, limiting the scale-up of mRNA production. Dr Rok Sekirnik and colleagues at Sartorius BIA Separations, Slovenia, found a way to monitor and analyse the production of mRNA in the laboratory while decreasing the cost by up to 50%.

CIMac™ analytical columns.

The COVID-19 pandemic was the first to introduce mRNA therapeutics on a massive scale to the whole world. Ever since, mRNA has played a pivotal role in many scientific and health domains, ranging from the development of vaccines for infectious diseases and treatments of genetic diseases to cancer immunotherapy. The production of mRNA, which consists of at least 11 main steps, is a laborious and costly process. That is why one of the main challenges is the development of approaches that would reduce the cost of mRNA production.

Dr Rok Sekirnik and colleagues at Sartorius BIA Separations, Slovenia, have successfully optimised a specific production step called in vitro transcription (IVT) to produce mRNA at a large scale while at the same time decreasing the cost of the final mRNA molecule dramatically.

THE mRNA REVOLUTION

Over the past years, scientists have been working on what we would now describe as the mRNA revolution, when they developed a new type of vaccine that uses a molecule called messenger RNA (mRNA) rather than part of a bacteria or virus. When the first two vaccines based on this novel technology – BioNTech/Pfizer's Comirnaty and Moderna's Spikevax – entered the market in 2020, they managed to revolutionise the field of biopharmaceuticals for good. Now, mRNA vaccines have been proposed to be used against a variety of targets, such as cancer, bacteria, and of course viruses, including HIV and malaria.

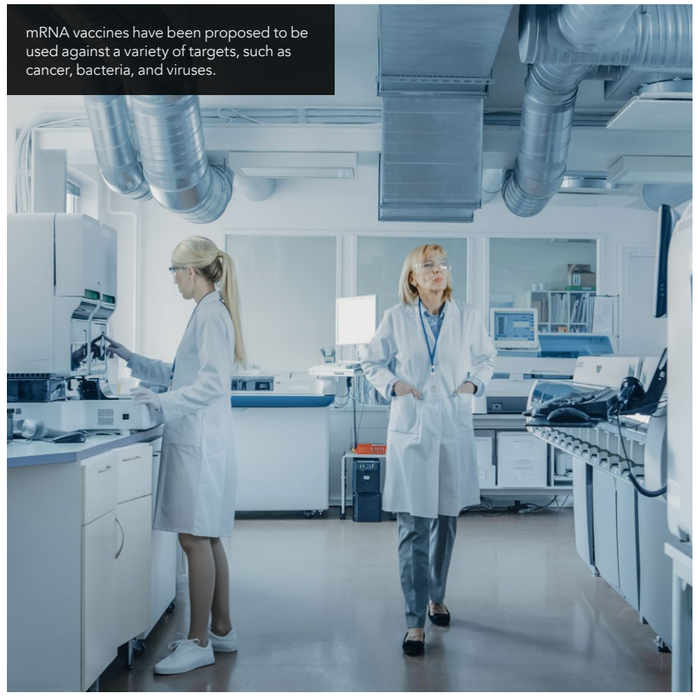
The novelty of the mRNA vaccines lies within their unique mode of action, which differs vastly from the one that typical vaccines have. Traditionally, vaccines contained a weakened virus or a piece of the virus's protein coat to trigger the immune response of our body and produce antibodies. On the other hand, mRNA vaccines work by introducing a piece of mRNA (genetic information) of a viral protein. By using this injected mRNA, the body starts producing the viral protein, which is later recognised by the immune system, thus initiating the immune response and finally the production of antibodies.

mRNA PRODUCTION METHODS

The production of mRNA occurs when one molecule of DNA is transcribed into multiple copies of RNA with the help of an enzyme called RNA polymerase. This enzyme takes the building blocks (called nucleotides) needed to create the mRNA and links them together to form a long chain, with the process resembling the creation of a LEGO design.

This reaction is rather expensive due to costly ingredients. Hence, ongoing research efforts are underway to reduce the cost of its production, focusing on the optimisation of the reaction. This includes controlling the number of building blocks available to create a complete chain and also the number of copies produced per one set of reagents (chemicals used in the reactions). Additionally, the mRNA is a highly challenging molecule to produce due to its large size and relative instability, complicating its production.

The process of producing an mRNA vaccine typically requires between 10 and 15 different steps, such as plasmid DNA production, plasmid linearisation, in vitro transcription (IVT) reaction, mRNA purification, and lipid nanoparticle (LNP) production. The IVT is the main reaction in which the RNA is produced from a DNA template. It's a step common to all existing methods to produce mRNA. In fact, the final cost of mRNA production depends on this particular step and on its yield. More specifically, during that reaction, a part called 'cap' is added to the mRNA molecule to achieve stability inside the human body – or, in other words, to achieve in vivo stability. Without the existence of the cap, the mRNA becomes vulnerable to enzymes, called exonucleases, which could mistakenly recognise the final mRNA molecule as a virus and destroy it. Unfortunately, the price of a capping reagent present during the IVT makes the whole mRNA production very expensive. To address this issue and reduce IVT cost, alternative methods have been proposed, including the possibility of continuous manufacturing of mRNA. Although a promising method, there have been no reports of a simultaneous removal of the mRNA product from the reaction. Therefore, there is a great need to design new methods to optimise IVT, ultimately reducing the overall cost of mRNA production and thus the cost of the mRNA vaccines.



mRNA vaccines have been proposed to be used against a variety of targets, such as cancer, bacteria, and viruses.

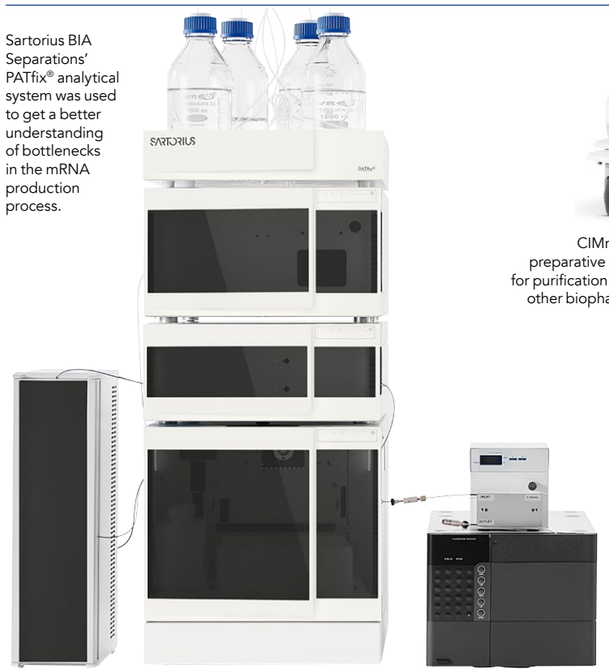
OPTIMISATION

Sekirnik and colleagues hypothesised that if they could monitor the IVT reaction, they might be able to increase the overall reaction yield. In other words, they tried to understand the rate at which the mRNA building blocks (reagents used in the IVT) are being consumed during the IVT reaction, so they could better predict when each of the reagents needs to be added to the reaction mixture. The team worked on optimising the yield of IVT reaction by developing a workflow that uses high-performance liquid chromatography (HPLC) to monitor the different components of the reaction mixture in near real time.

By using a particularly fast chromatographic technology called monolith chromatography (CIMac PrimaS) and a dedicated PATfix® analytical system mRNA HPLC platform, they managed, in less than three minutes, to determine the concentration of the different reagents important for the reaction, including the concentration of nucleoside triphosphates (NTPs) – the mRNA building blocks, capping reagent, plasmid, and mRNA. Practically, this allowed the group to understand several important parameters influencing the yield of the reaction, such

The production of mRNA is a laborious and costly process which consists of at least 11 main steps.

Ongoing research efforts are underway to reduce the cost of mRNA production.



Sartorius BIA Separations' PATfix® analytical system was used to get a better understanding of bottlenecks in the mRNA production process.

CIMmultus® line of preparative columns used for purification of mRNA and other biopharmaceuticals.

The group's approach led to a cost reduction of 50% per mg of mRNA produced.

as when a reagent needs to be added to the reaction mixture or when the reaction is finished.

This workflow allowed for the implementation of a fed-batch approach (where a reagent is added to a reaction mixture throughout the duration of the reaction and not just at the start of the reaction), resulting in doubling the reaction yield compared to standard IVT protocols, reaching the final production yield of over 10 mg/mL. Sekirnik and coworkers also applied this HPLC-based monitoring concept to successfully produce therapeutically relevant mRNA lengths (which typically consist of several hundred nucleotides up to several thousand), demonstrating that the workflow they developed has immediate applications to the production of mRNA biopharmaceuticals.

SCALING UP THE PRODUCTION

Following the IVT optimisation, the group set out to use their approach to produce a larger amount of mRNA using a bioreactor. They combined their monitoring technique with a large, single-use reactor (AMBR250), which is widely used in many biopharmaceutical laboratories for cell-based production of biopharmaceuticals. This approach managed to produce approximately 2 g of mRNA from a relatively small reaction volume of 100 mL, which is sufficient to produce approximately 20,000–60,000 vaccine doses (if we assume the dosing used by BioNTech/Pfizer and Moderna in the COVID-19 vaccine). Additionally, this approach led to a cost reduction of 50% per mg of mRNA produced compared to batch mode production.

Now, the next step for the group would be to work on automated sampling, coupled with data analysis to manage and minimise impurity formation, and at the same time using computational tools to analyse large data sets on IVT reaction kinetics.

In conclusion, automated bioreactor systems used in a fed-batch mode, coupled with a PATfix® HPLC-monitoring system, could initiate the large production of mRNA, which will decrease the cost of reagents and finally decrease the cost of the mRNA vaccines. Sekirnik and colleagues managed to give an answer to a frequently asked question: can we produce large batches of mRNA with limited cost? This could improve the production of mRNA molecules and lead to the development of mRNA vaccines for many different diseases.

The workflow the researchers developed has immediate applications to the production of mRNA biopharmaceuticals.

www.researchoutreach.org

Behind the Research

Dr Rok Sekirnik

E: rok.sekirnik@biaseparations.com **W:** www.biaseparations.com

Research Objectives

Dr Sekirnik and his team at Sartorius BIA Separations optimised a crucial step in the mRNA production process, resulting in a higher yield.

Detail

Bio

Rok Sekirnik graduated in chemistry from University of Oxford where he also completed his doctorate. He has since worked in analytical and process development for production of biopharmaceuticals, including monoclonal antibodies, virus vaccines, and mRNA and held roles in Novartis, Batavia Biosciences and Sartorius BIA Separations, where he is Head of Process Development for mRNA/pDNA.

Funding

Sartorius BIA Separations, d.o.o.

Collaborators

- Janja Skok
- Polona Megušar
- Tina Vodopivec
- Domen Pregeljc

Competing interest statement

Rok Sekirnik is an employee of Sartorius BIA Separations.

SARTORIUS

References

Pregeljc, D, et al, (2023) Increasing yield of in vitro transcription reaction with at-line high pressure liquid chromatography monitoring. *Biotechnology and Bioengineering*, 120(3), 737–747. doi.org/10.1002/bit.28299

Skok, J, et al, (2022) Gram-scale mRNA production using a 250-mL single-use bioreactor. *Chemie Ingenieur Technik*, 94(12), 1928–1935. doi.org/10.1002/cite.202200133

Personal Response

What did you find most exciting about this work and where do you see the field moving in the future?

" The COVID-19 pandemic revealed both the tremendous clinical potential of mRNA vaccines and the need for innovation in production technologies to produce cheaper vaccines of high quality at large scale. We wanted to better understand the bottlenecks in the process and realised that not enough attention was paid to the most critical step in production. By applying our analytical technology, we gained valuable insights into the reaction that produces mRNA; this led to better control of the reaction and consequently an ability to reduce the cost of producing mRNA. We continue to innovate in developing better bioprocessing tools and data processing technologies to get a better understanding of the mRNA production process. I am very excited about the near future – there will be lots of innovative technological developments in the mRNA field. "