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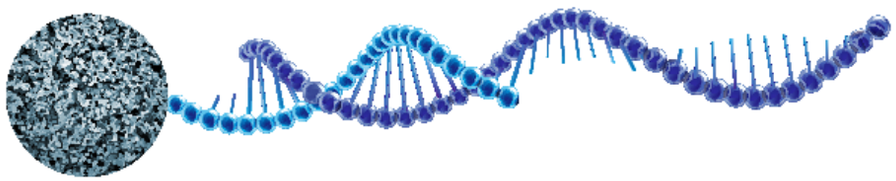
GET IN THE mRNA VACCINE RACE WITH AFFINITY PURIFICATION

On March 16, 2020, the biotechnology company Moderna Therapeutics announced that the first person in a Phase I clinical trial had been dosed with its messenger RNA (mRNA) vaccine against SARS-CoV-2, the virus that causes COVID-19. The company reached this milestone just 63 days after Chinese authorities published the genetic sequence of the virus, SARS-CoV-2.

Developing any new therapy or vaccine from conception to clinical use is a marathon rather than a sprint. But in 2020, the research teams developing mRNA vaccine technology against SARS-CoV-2 have rocketed out of the starting blocks.

With demand for the large-scale production of clinical-grade mRNA suddenly surging, developers need fast, efficient, and highly scalable methods for mRNA purification. The bench-scale mRNA purification methods used until now are becoming a significant bottleneck to large-scale manufacture. Thermo Fisher Scientific has developed a new affinity-based mRNA purification product, Thermo Scientific™ POROS™ Oligo (dT)25 affinity resin, tailor-made for scalability. The mRNA binds selectively to the surface of the Oligo (dT) beads, and any impurities are simply washed away.



Schematic of Thermo Scientific™ POROS™ Oligo (dT)25 affinity resin, consisting of porous polymer beads coated with deoxythymine (dT) strands that can capture mRNA's poly-A tail.

Image credit: Thermo Fisher Scientific

The speed with which mRNA vaccine candidates can be designed and produced is a key advantage of this new vaccine technology. Traditional protein-based vaccines are inherently time-consuming to produce, even when using the latest cell culture techniques. To date, the shortest vaccine development pathway has been 5 years for the protein-based Ebola vaccine, Ervebo.[1] On April 14, 2020, when two giants of protein-based vaccine production, GlaxoSmithKline and Sanofi, announced that they would collaborate to develop a SARS-CoV-2 vaccine, they forecast that human clinical trials would have to wait until the second half of 2020.

The SARS-CoV-2 crisis could springboard the first mRNA vaccine into widespread use. Even before the disease, mRNA vaccine candidates against pathogens, from Zika virus to rabies, were showing promise in human clinical trials. Aside from vaccines, a variety of mRNA-based therapies are beginning trials for health conditions ranging from common cancers to rare genetic disorders. In May 2019, market analysts at Visiongain forecast that the mRNA vaccines and therapeutics market would grow at a compound annual growth rate of 9.18% between 2019 and 2029, reaching a market value of up to $8.90 billion.[2]

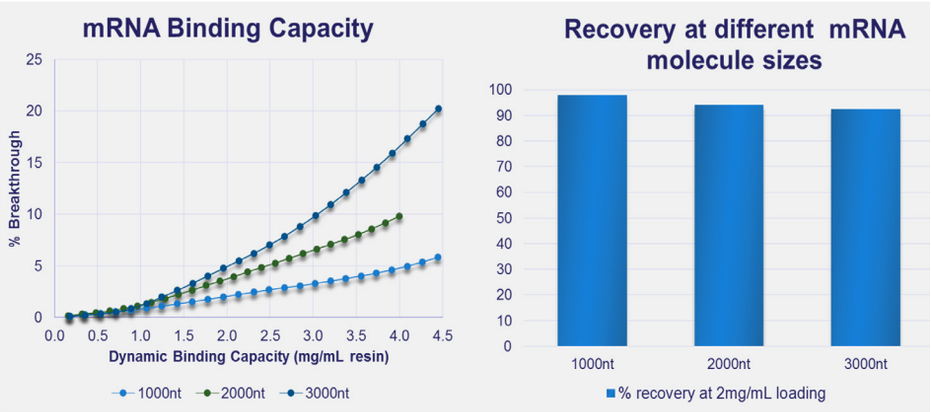
Affinity-based scalable mRNA purification removes one bottleneck to the rapid rollout of mRNA vaccines and treatments for otherwise unstoppable diseases.

VACCINES

Vaccines based on mRNA are making steady progress through clinical trials toward regulatory approval and clinical use, says Piotr Kowalski, an mRNA researcher at University College Cork. “I think the first clinical applications of mRNA will clearly be vaccinations,” he says. “mRNA is well suited towards that application.”

Particularly in light of the global SARS-CoV-2 research effort, mRNA vaccine production is where the need for high-throughput mRNA purification is likely to become the most acute. With mRNA vaccines and therapeutics, the aim is to piggyback onto the body’s own protein-producing mechanism to gain a health benefit. The natural role of mRNA is to relay protein sequence information from cells’ DNA to the protein-producing ribosomes. When researchers slip synthetic mRNA into cells, the ribosome will produce the corresponding protein.

In the case of vaccines, mRNA is selected that encodes a protein from a virus. Once the injected mRNA is taken up by cells, the body churns out copies of this protein, and the immune system learns to recognize it as an antigen. Many of the mRNA vaccine candidates for SARS-CoV-2 encode proteins that make up part of the virus’s characteristic spiky surface. With a successful vaccine, if a vaccinated person later becomes infected with SARS-CoV-2, the immune system would be trained to recognize and fight it.



Dynamic binding capacity of the resin is influenced by the size of the mRNA construct size (left), while recovery is size independent (right).

Image credit: Thermo Fisher Scientific

Currently, traditional vaccine makers manufacture the antigen protein itself—a cell-based process that takes months to set up and run. "With mRNA vaccines, the patient's own body becomes the antigen factory," Kowalski says. As mRNA can be rapidly manufactured in a cell-free system, mRNA vaccines are faster to produce than protein vaccines.[3] The switch to affinity purification—already used to purify protein-based biologic drugs at large scale – should further streamline the process.

There are several other reasons mRNA lends itself to vaccination, Kowalski says. Protein-based vaccines generally require a second ingredient, known as an adjuvant or excipient, to boost the immune response and help ensure the antigen generates strong, long-lasting immunity. With mRNA, however, the immune system is inherently on the lookout for it because many viruses are RNA-based. "The mRNA is a natural adjuvant, which helps to boost the immune response," Kowalski says.

The body's virus surveillance systems can be exploited in further ways to boost mRNA vaccine efficacy, says Harry Al-Wassiti, who is developing mRNA-based vaccines and therapeutics at Monash University. He and his colleagues have been researching the lipid nanoparticles used to encapsulate mRNA prior to injection. "The nanoparticles have two roles: to protect the mRNA and to deliver it to cells," Al-Wassiti says. For vaccines, immune cells called antigen-presenting cells (APCs) must see the antigen to elicit the immune response. "From the surface, these lipid nanoparticles look almost like a virus, so the APCs take them up," he says. The Monash team and others have been developing lipid nanoparticles that APCs recognize particularly effectively.

Because of these theoretical advantages, several companies are pursuing mRNA vaccines. "We know we can develop and manufacture them much quicker than conventional vaccines—the question is how well they will work," Kowalski says. "Everybody is looking for the clinical data, and so far, the data looks promising." For example, Moderna also has an mRNA vaccine in Phase II clinical trials for

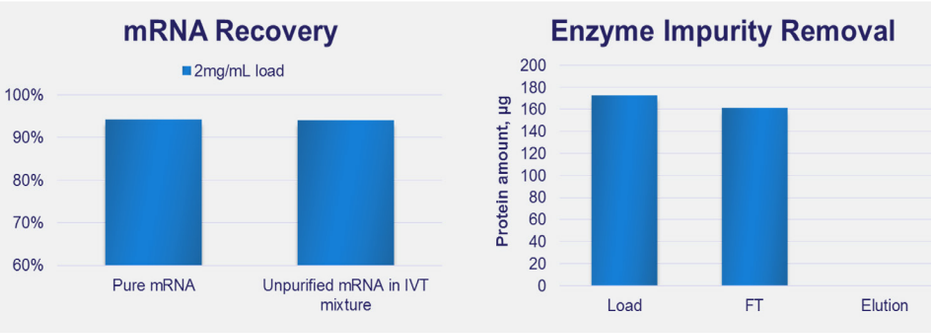
cytomegalovirus, a leading cause of birth defects. The company has conducted Phase I trials on eight other mRNA candidate vaccines, including against the H10N8 influenza virus and H7N9 avian flu virus.[4]

The mRNA company CureVac, based in Tübingen, Germany, has a rabies vaccine in Phase I clinical trials and recently announced that the vaccine elicited a strong immune response with two shots of a microgram mRNA dose. "That's a really low dose compared to regular vaccines, so there is a lot of promise," Al-Wassiti says. It's another shot in the arm for the efficacy of mRNA vaccines. CureVac is also actively working on a SARS-CoV-2 vaccine, as are Al-Wassiti and his colleagues.

Blocking infections isn't the only application of mRNA-based vaccines, which are being explored and tested for a number of other conditions. Cancer is one of the most actively explored areas. "Instead of a virus, you identify the tumor antigen and try to train the immune system to go after the tumor," Kowalski explains. The immune system learns to recognize and kill the cancer cells. If approved, a cancer vaccine would require large-scale mRNA production and purification. Both Moderna and CureVac have clinical trials underway for vaccinations against cancer.

TREATMENTS

Vaccines are not the only mRNA-based interventions that could direct the immune system to attack a person's cancer, Kowalski adds. As a postdoctoral researcher at the Massachusetts Institute of Technology, he used mRNA to generate antibodies as a cancer therapy. Antibody proteins, such as trastusumab (Herceptin), are already used as therapeutics against some cancers. Kowalski and his colleagues demonstrated that there could be health benefits to dosing patients with the trastusumab mRNA and having the body make the antibody.[5] "Injected antibodies are often quickly cleared from the body," he says. But with mRNA, the body continually produces the protein for a period, somewhat like a slow-release version of the antibody. "We showed we could express the antibody directly in the mouse, to achieve a better pharmacokinetic profile and a better efficacy in terms of treating cancer," Kowalski says.



Unpurified mRNA in an in vitro transcription (IVT) mixture shows similar recovery to loading pure mRNA onto the columns, meaning impurities the in feedstock do not have an impact on final recovery (left). Using the POROS Oligo (dT)25 resin >99% of the protein impurities is removed from the IVT feedstock. Levels of protein in the elution pool are below detection limits.

Image credit: Thermo Fisher Scientific

Another advantage of mRNA therapeutics compared with proteins is that, from a purification standpoint, all mRNA molecules are essentially identical, Al-Wassiti says. That's because the amino acids in proteins are chemically diverse, while the ribonucleic acids that make up mRNA are relatively similar. "Whereas protein purification really varies depending on the protein sequence, all mRNA looks the same, so the way you purify, it is the same," Al-Wassiti says. It's therefore likely that large-scale mRNA affinity purification protocols pioneered for vaccine development could be readily adopted for mRNA therapeutics.

mRNA also has a number of potential applications in gene therapy. For instance, genetic disorders in which people lack or produce defective copies of a particular protein may be prime targets for mRNA treatments. People with cystic fibrosis, the most common fatal inherited disease in the US, lack a functional copy of the cystic fibrosis transmembrane conductance regulator (CFTR) protein, leading to mucus buildup in the lungs and other organs. By dosing with the relevant mRNA, the body can produce functioning copies of CFTR protein.

Some of the biggest challenges of mRNA protein replacement therapies concern delivery and safety, Kowalski says. The key is to get the mRNA into the cells that need it, while avoiding other cells where expressing the protein could have a deleterious effect. But certain areas of the body already can be targeted selectively. For example, lipid nanoparticles loaded with therapeutic mRNA can be targeted to the liver, where a range of diseases could be treated. For cystic fibrosis, inhaled formulations designed to deliver mRNA directly to the lungs are being developed.[6] Lexington, Massachusetts–based Translate Bio is testing an inhaled mRNA cystic fibrosis therapy in a Phase I/II clinical trial.

Complementing the protein replacement work, mRNA is well suited to knock out specific genes. The therapeutic mRNA can encode the expression system for the gene-editing tool CRISPR-Cas9. "With CRISPR-Cas9 we can also use mRNA to efficiently turn off gene expression," Kowalski says. Again, delivery is a key challenge and one of the major areas of research focus for his group. Once ready for scale-up, mRNA-based gene-silencing products could benefit from the high-throughput affinity purification protocol pioneered in areas of mRNA research that are a step or two further along the path to clinical use.

MEETING DEMAND

As multiple mRNA drug and vaccine candidates progress from bench-scale research toward clinical use, the demand for larger quantities of clinical-grade mRNA is increasing rapidly. "Therapeutic functional messenger RNAs are a really hot topic and a dramatic driver of growth for us," says Peter Scheinert, CEO and founder of AmpTec, a Hamburg, Germany–based contract manufacturing company of current good manufacturing practices–grade synthetic nucleic acids for therapeutic and diagnostic applications. "In the past year, we have doubled the number of employees in this area," he says.

When Thermo Fisher approached AmpTec in 2019 to test a new affinity-based mRNA purification product specifically designed for large-scale applications, the company was very receptive to the idea, Scheinert says. “We were very excited, because it is very important for us to actively prepare for the increasing requests and demands from the market regarding scale,” he says. “mRNA production scales will certainly increase, and we need purification options available that can deal with large scales.”

Until now, most manufacturers have used reverse-phase high-performance liquid chromatography (HPLC) to purify mRNA. But that has two major limitations, according to Scheinert. At small scale, HPLC offers very high-resolution separation of components in a reaction mixture. As scale increases, larger column beads are necessary to prevent the pressure from becoming too high, which could compromise separation performance. In addition, scaling up reverse-phase HPLC requires larger volumes of toxic organic solvents, such as acetonitrile, which have negative health and environmental effects.

AmpTec had therefore already ruled out HPLC and was using alternative mRNA purification options, Scheinert says, but the technique is only suitable for purifications of up to 1–2 grams. “For larger scale, there is a lot of hands-on time, which is not suitable for scales of 10 grams, 20 grams, or larger,” he adds.

Thermo Fisher customers had been requesting custom solutions for large-scale mRNA purification, according to Scott Zobbi, the firm’s business lead for custom POROS chromatography resins. “When three or four people start to ask you the same thing, you realize there’s a broader demand,” he says. Kelly Flook, its senior product manager for bioprocess purification resins, led the development of an mRNA affinity purification product that would be available to all customers: POROS Oligo (dT)25 affinity resin. AmpTec is among several mRNA manufacturing companies that have helped put the new product through its paces.

Oligo (dT)25 resin leverages the fact that all mRNA molecules, natural and synthetic, feature a poly-A tail, a stability-enhancing chain of adenine nucleotides at one end of the molecule. The product, which exploits complementary base pairing between adenine and thymine to isolate mRNA after synthesis, consists of porous polymer beads coated with deoxythymine (dT) strands that can capture mRNA’s poly-A tail.

At the end of an mRNA synthesis, the reaction mixture is combined with a sodium chloride solution, then loaded onto a column filled with Oligo (dT) beads. The sodium ions in the salt solution neutralize the negative charges found along the backbone of the RNA molecules; that allows the poly-A tail to form hydrogen bonds with the dT strands on the beads. The impurities from the reaction mixture are simply washed off the column when flushed with more of the salt solution. When the column is flushed with fresh water, the sodium ions are then washed away. The negative charges on the backbones of the Oligo (dT) and the poly-A tail repel each other, breaking the base pairing and releasing the now purified mRNA. “Within a couple of column volumes, you will have collected a purified, concentrated solution of your target mRNA,” Flook says.

Part of the product's appeal is that dA-dT affinity binding is a tried and true method for purifying mRNA samples at bench scale, Scheinert says. The new product transfers the Oligo (dT) coating to a 50 µm polystyrene divinyl benzene cross-linked porous bead. Compared with typical HPLC resins, the bead is large, according to Zobbi. "But because it has inherent porosity, you have an increased surface area," Zobbi adds, which means greater capacity to bind mRNA. And as it's a bead-based product, users have the flexibility to pack it into a column of any size. They can tailor the purification step to the scale of the mRNA sample to be purified.

Basing Oligo (dT) on the proved POROS bead technology inspires confidence in the product, says Joseph Barberio, the director of process development at Strand Therapeutics, a seed-stage biotech company developing programmable mRNA therapeutics based in Cambridge, Massachusetts. "POROS resins are proven at scales from benchtop to commercial manufacturing operations," says Barberio, who has tested the new resin. "Utilizing the same technology from early development through scaled manufacturing is key to the successful tech transfer and execution of a manufacturing campaign."

Barberio says his experience with Oligo (dT)25 resin has so far been positive. "For so long, the RNA sector has been working with resins that were not made for RNA. It is really great to see a major manufacturer focused on designing products for the mRNA space."

Scheinert was also gratified by the move. "I was very happy to hear there would be a large-scale option for Oligo (dT) purification," he says.

With large-scale orders from customers expected soon, AmpTec is prioritizing the switch to POROS Oligo (dT)25 affinity purification, Scheinert says. The company is likely to adopt the product for all mRNA purifications, he adds. "It would make sense to have one purification method that applies at all scales, in order to have consistent product quality. It is really very flexible, so it would be a good idea to use it exclusively for all mRNA batches—large and small."

The development of mRNA vaccines and therapeutics is a very active space, with progress being made on multiple fronts. The advancements are being further driven by SARS-CoV-2. "There is so much basic research coming out, a lot more open access publishing and data sharing," Kowalski says. "It is an unprecedented crisis, and the response of the scientific community is also unprecedented." Mastering large-scale mRNA purification may be the key to producing a future therapy against SARS-CoV-2 and, with so many mRNA products in development, many other diseases as well.

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