GETTING SAFELY TO THE DESTINATION

Liposomes transport active pharmaceutical ingredients to the target cells, while ensuring that the cargo does not break down prematurely during its journey through the body. Their use opens up new therapeutic pathways for the treatment of cancer as well as genetic and other diseases.

TEXT **NIELS BOEING**

Biologists continue to dig deeper and deeper into the composition and functionality of cells to better understand their enormously complex biochemical machinery. Through this process of scientific discovery, they decipher the importance of more and more genes in cell metabolism and discover new pathways. Pharmaceutical companies hope to use this new knowledge to develop new therapies against cancer, hereditary diseases, and other serious illnesses. In the process, they are increasingly turning to ribonucleic acids (RNAs for short) with the goal of knocking out specific genes or promoting the formation of essential proteins.

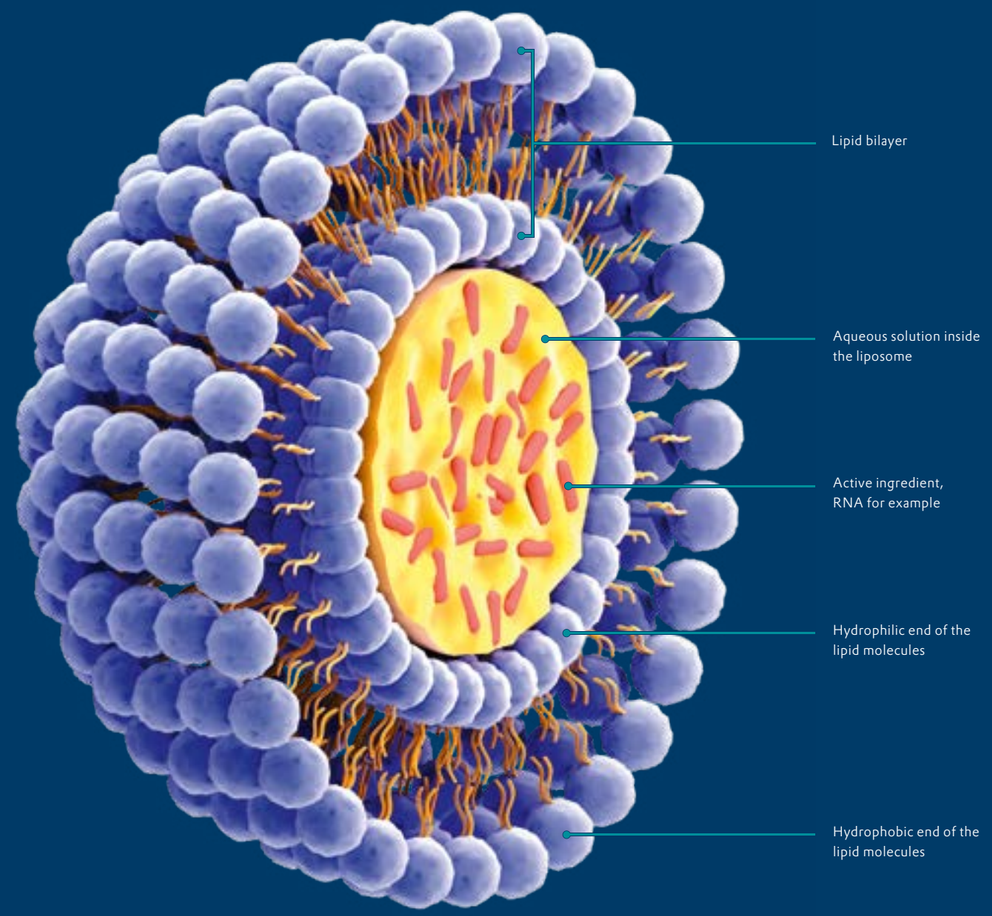
For this to work, the RNA molecules must make their way directly to the targeted cells of the affected tissue. "RNA strands are unstable," says Andrea Engel, who is responsible for drug delivery technologies in the Innovation Management unit of the Health Care business line at Evonik. If they were injected directly into the bloodstream, they would be broken down rapidly by the immune system. So there is a need for transport mechanisms that can convey their pharmaceutical package directly into the interior of the cells.

Liposomes provide one of the most important mechanisms to achieve this outcome. They are tiny spherical structures with outer membranes composed of a bilayer of membrane-forming molecules such as phospholipids or fatty acids, and they can be loaded with RNA. Unlike tablets or capsules, however, liposomes cannot be administered via the digestive tract. There, the RNA-charged liposomes would simply be broken down. Instead, they have to be administered by a parenteral route such as via intravenous injection directly into the bloodstream.

A COMEBACK THANKS TO NEW THERAPIES

Liposomes have actually been known for a long time. The British physician Alec Douglas Bangham discovered them in 1964. The first approvals for liposomal formulations of active ingredients for use as medications were issued almost three decades ago. Ambisome, the first remedy to contain an active ingredient against fungal infections that was formulated using liposomes, was launched on the market in 1990. This was followed in 1995 by Doxil, a preparation using liposomes charged with an active ingredient against Kaposi's sarcoma, a type of cancer that forms in the lining of blood and lymph vessels. Doxil was the first cancer medication of this type, and further indications followed. →

A liposome is created when a double layer of lipids arranges itself into a ball due to molecular interactions. Water-soluble active ingredients can be stored inside the liposome, which can then transport them to their destination



The cosmetics industry was increasing its use of these formulations in parallel. While liposomes developed into a standard technology in that area after 2000, their applications for medical use were less visible. However, this has now begun to change.

"Liposomes are making a comeback, because they are so well-suited to new therapeutic applications with strands of RNA or DNA," says Stefan Randl, who is responsible for Innovation Management at Evonik's Health Care business line. "The liposomes can be manufactured in an extremely flexible and customized way, and can thus be applied for a wide range of active ingredient formulations."

With liposome-based RNA therapies helping to create new segments in the pharmaceutical market for advanced drug delivery, Evonik acquired Transferra Nanosciences, a leading contract development and manufacturing company for liposomal active ingredient formulations, in 2016. This Canadian company, which is based in Vancouver and is now called Evonik Vancouver Laboratories, has also developed a technology that enables liposomes to be produced with extreme precision in a narrow, well-defined range of sizes.

FASCINATING PROPERTIES

One option for the production of liposomal active ingredient formulations involves a special extrusion process. First of all, an ethanolic solution of lipids, which are molecules that also occur naturally as the building blocks of cell membranes, are mixed with an aqueous phase in which the active pharmaceutical ingredient is present. During the mixing, tiny structures arise in which the lipids create a bilayer. Lipids are long molecules that have one hydrophilic (water-attracting) end and one hydrophobic (with little affinity to water) end. In aqueous systems, they line up parallel to one another and form a ball filled with water. The membrane that forms the outer shell of the liposome is a lipid bilayer, which is a double layer of lipids that is similar to the membrane surrounding a cell. This is one reason why researchers were fascinated by the discovery of liposomes. During the production of the formulation, medically active ingredients that are not soluble in water accumulate preferentially in the lipid bilayer, while water-soluble ingredients accumulate in the interior of the sphere.

> "Liposomes are making a comeback"
>
> STEFAN RANDL, INNOVATION MANAGEMENT
> EVONIK HEALTH CARE

The lipid structures with their active ingredient loading are then forced through a membrane with pores of a specific diameter in the range of one hundred nanometers, i.e. one hundred times smaller than the diameter of many of the cells that comprise human tissue. The liposomes form when they pass through the membrane. As a result, the original dispersion of liposomes becomes more homogeneous and the size distribution grows narrower. After the extrusion processes, the liposomes produced are all of similar size—around the same diameter as the pores in the membrane.

"The advantage of this process is that it can easily be scaled to clinical and commercial production," says Randl. A broad range of batch sizes, from a few milliliters to hundreds of liters, can be produced using the extrusion process at Evonik Vancouver Laboratories. To illustrate, a batch of 100 liters can theoretically provide up to 10,000 ten-milliliter doses, which is the standard dose of the medication Doxil. Thanks to the use of membranes with different pore sizes, the process can produce liposomes with diameters ranging from 50 to 200 nanometers.

CAMOUFLAGE AGAINST THE BODY'S DEFENSES

RNA strands are copies of individual genes (refer to the information box). Similar to the punched cards that were used with early computers to store data, the sequence of their nucleobases is evident in the interior of the cell. They can determine which amino acids are linked together, and which proteins (which essentially control the cell's metabolism) are formed.

In cancer cells, the genome has mutated so that the individual genes are permanently issuing instructions to the cell that it is to divide, and thus grow a tumor. Likewise, in hereditary diseases genes with defects are no longer transcribed, resulting in metabolic or other disorders. Because RNA strands carry the instructions for these processes, scientists recognize that RNA interference technologies may provide solutions and enable causal treatment of the disease. If RNA strands with nucleobase sequences that are complementary to those of the cell's own RNA are introduced, the cellular RNA is inhibited. These RNA strands, which are known as siRNA (small interfering RNA), can suppress the action of the genes transcribed by the RNA.

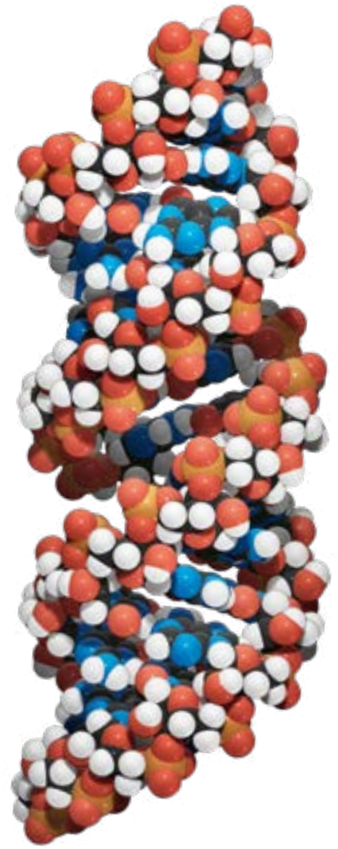
Liposomes can be used to transport siRNA into the cells. The liposomes provide the siRNA with a kind of protective coating that enables them to be transported without being noticed by the body's immune system. The liposomes can reach their target cells because the blood vessels in tumor tissue are not properly lined up, as in other tissue, but have gaps. The liposomes can thus diffuse out of the bloodstream into the pathological tissue and make their way further into the cells.

Various pharmaceutical companies have developed siRNA that can destroy carcinogenic liver cells or reduce the uncontrolled cellular division of tumor cells. "Some RNA liposome preparations are already in phase III clinical studies," says Engel. It is therefore likely that therapies of this type will become part of the range available for treating patients in the coming years. The first liposomal RNA medication was recently approved by the US pharmaceuticals authority. Onpattro® will in the future be used for the treatment of familial amyloid polyneuropathy, a hereditary disease which leads to symptoms including signs of paralysis and muscle loss in the extremities. With the active ingredient molecule inhibiting the abnormal production of the protein that is responsible for the syndrome, the therapy promises to substantially improve the quality of life for affected patients.

A MARKET WITH PROSPECTS FOR GROWTH

Similarly to the RNA process, liposomes can also be used to transport genes, which are strands of DNA, into a cell. That makes lipid vesicles of interest to those seeking to create new types of gene therapy. Randl therefore expects to see considerable growth in the market for RNA and DNA therapies over the coming decade.

However, the first generation of RNA and DNA therapies has not yet been fully exploited within this market segment. Many research institutes around the world are working on a further improvement that is known as active drug targeting. Here, transport systems, such as liposomes, are augmented by the addition of other molecules which bind to receptors on, for example, tumor cells. The objective is to prevent liposomes from getting lost on the way through the bloodstream, and to deliver them in complete form to the target tissue. In the future, the use of such precise drug targeting in precision medicine will open up new treatment options that are far beyond what is possible today. ▬



Messenger RNA is a long, twisted molecule that copies part of the DNA and thus transports the genetic information required for protein synthesis

Nucleic acids

Nucleic acids such as DNA and RNA are molecules that are essentially composed of nucleobases. In DNA, these are adenine (A), thymine (T), cytosine (C), and guanine (G), and are arranged in the pairs A-T and C-G. This gives rise to the typical double helix of DNA, a twisted ladder with A-T or G-C base pairs as the rungs. RNA contains uracil (U) in place of the thymine in DNA. The sequence of the nucleobases in the DNA encodes the gene. Each set of three base pairs in the gene codes for a specific amino acid that is added to the protein being synthesized in the ribosomes. Messenger RNA is a single-strand copy of the sequence of the DNA, with uracil standing in for the thymine. This copying process is called transcription. The RNA transfers the information in the sequence of base pairs from the gene to the ribosome. In addition to the important messenger RNA, the cell also contains other RNA strands with other functions such as ribosomal RNA or transfer RNA.