Targeted Drug Delivery: From Science Fiction to Reality

Professor Richard L. Klemke

Scientia

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Most human diseases are localised in terms of their location but currently, injected or orally administered drugs are evenly distributed all over the body and thus, act indiscriminately. The targeted delivery of medication to the exact site where it is needed is a common theme in science fiction but thanks to **Professor Richard Klemke** and his team at the University of California San Diego's Moores Cancer Center, this fantasy may soon become a reality.

The Fiction

Most of us will be familiar with the recurring scenario in science fiction: the protagonist, struck down with some fatal disease or injury, is saved miraculously by an ingenious drone programmed to navigate through the blood vessels, arrive at the site of the problem, and release the necessary drug just in time. Our hero survives another day. But is there any element of truth in this popular storyline that holds such promise for the future of medicine?

The Reality

The reality is that our ability to deliver drugs to precisely where they are needed in the body is very limited. The main problem is that most diseases are localised. Cancer, for example, usually affects only one site. Arthritis, most infections, diseases of the nervous system, and, in fact, just about any disease you can think of, will involve either one site or a limited number of select locations. Yet, in the absence of a better option, drugs are administered systemically. Irrespective of whether taken orally, injected into the blood, into a muscle or under the skin, medication is absorbed, circulates in the blood, and is deposited indiscriminately, everywhere in the body.

This approach has two significant shortcomings. First, a very large proportion of the drug ends up in places where it is not needed. This is not only wasteful but can be outright damaging for the patient. Take cancer again as an example. In an ideal world, we would want all cancer-killing chemicals concentrated within the tumour to provide the best chance of a cure because when the drug goes everywhere, it also affects other tissues. Problematically, cancer-killing chemicals in the bone marrow can switch off the production of red blood cells, causing anaemia. Elsewhere, damage to cells lining the gut can cause nausea and weight loss. An unwanted effect in the skin is hair loss. Other vital organs, the kidneys, the heart muscle, nerves, and hormone-producing organs can all be affected by indiscriminate drug distribution, causing severe side effects.

For these reasons, physicians try to delicately balance the dose of medication to ensure that it is suitably effective while also, minimising

unwanted side effects. Unfortunately, such compromises limit our ability to treat many serious diseases effectively. Although researchers have tried several methods to overcome these problems (for example, utilising nanotechnology and microscopic fat droplet-mediated drug delivery), the results have usually been poor.

Starting with Mesenchymal Stem Cells

Professor Richard Klemke at the University of California San Diego's Moores Cancer Center is a world leader in this exciting field. He explains that the starting point for his work was a fairly simple observation regarding mesenchymal stem cells (MSCs) involved in tissue regeneration. For example, when we cut a finger, MSCs

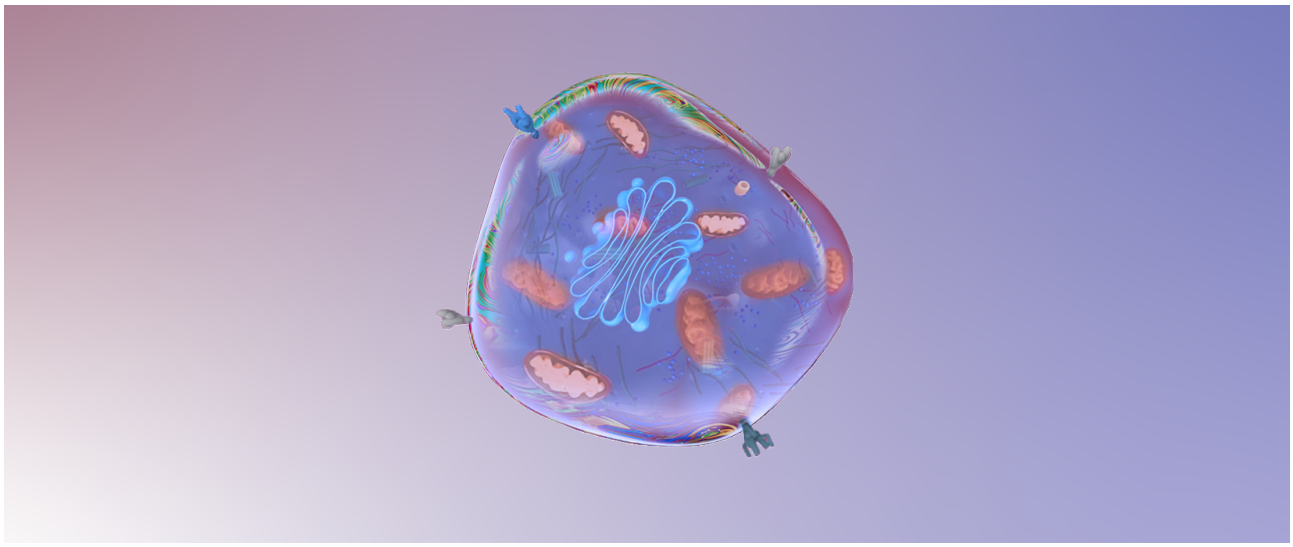

Illustration of a CargocyteTM. Reproduced with permission from Richard Klemke.

move to the site of the injury and repair the damage by turning into cells that are needed locally and releasing beneficial substances.

MSCs caught Professor Klemke's attention because here, he noted, is a cell that can move, can find its way to an injury, and can release large amounts of chemicals on the spot. Was there a way to utilise these MSCs for the targeted delivery of specific drugs?

Theoretically, it all seemed possible although there were considerable challenges to be overcome. For a start, MSCs repair damage by turning into other cells that are lost during the initial injury and uncontrollably produce unwanted factors. This ability is not only unnecessary for the purposes of targeted drug delivery but is also potentially damaging. As such, the accumulation and long-term survival of such cells and their secreted products could alter structures or cause other harm.

The genetic programme necessary for these unwanted effects is coded in the nucleus of the MSCs. Professor Klemke saw that the solution to several problems associated with using nucleated cells as therapeutic delivery devices was simply to remove the dangerous DNA from the cell. By removing the entire nucleus through a process known as cell enucleation, he reasoned that the cell would be rendered safe with a lower potential for carcinogenicity. Cell enucleation would also block new gene transcription, preventing the induction of potentially unwanted products after the cell was introduced into the body. Finally, the enucleated cell would have a defined life span which would make drug delivery more controlled and predictable.

But key questions remained, including the issue of how long the cells would survive after enucleation, whether they would still perform the critical migration functions necessary for locating and physically moving into disease tissues, and whether they would produce and deliver a defined therapeutic at the site of disease as intended. But, if the enucleated cells could survive and perform as hoped, then possibly, they might act like delivery drones dedicated to bringing defined therapeutics to diseased tissues in a much safer, controlled, and predictable manner than previously possible.

Professor Klemke's team developed the necessary technology to achieve this by optimising the conditions for gently removing the nucleus from the cell. The resulting structures could no longer be considered cells, as they couldn't divide, survive for long, or develop independently. Due to their intended use, the team decided to call these nucleus-free, cargo-carrying cell-derived particles Cargocytes™. The removal of the nucleus meant that most of the regulatory concerns and risks associated with cell therapy would no longer apply, making it easier to gain regulatory approval for their use in the future.

Professor Klemke and the team undertook rigorous functional testing to determine the therapeutic utility of the enucleated cells using animal systems – and this worked pretty well. But excitingly, the researchers knew they could substantially improve this process even further by introducing a GPS-like system to guide cells to diseased tissues using gene and cell engineering technologies.

The Guidance System

While Cargocytes™ have the necessary machinery to move, their use for drug delivery required a way to guide them to the location where they are needed. The biology behind this is complex. Imagine that there is an infection in your right earlobe, while Cargocytes™ intended to treat this infection are swimming around in your blood, inside blood vessels. How would a Cargocyte™ 'know' where to come out and deposit its content?

Cancer on healthy tissue.

What helps is that there is a signal coming from the infected earlobe, alerting other cells to the problem. Biologists refer to these signals as chemokines. The first thing the team needed to do was to provide the Cargocyte™ with the ability to detect these signals. Molecules with this sensory function are called chemokine receptors. Perhaps it is easiest to imagine these as antennae on the Cargocyte™ surface, allowing it to detect the presence of a chemokine.

Once the Cargocyte™ reaches the location where the chemokine signal is the strongest, there needs to be a mechanism that prevents it from being swept along with the blood flow. Rather, it needs to stop and then move out of the blood vessels passing near the diseased tissue. There are two types of molecules involved in this process: adhesion molecules and selectins. Adhesion molecules can be thought of as 'hands' holding onto selectins, microscopic 'grab handles'. Adhesion molecules attaching themselves to selectins allow the Cargocyte™ to stop and 'climb' in the required direction.

During initial experiments, Professor Klemke and his team were able to insert three different chemokine receptors – 'antenna' – onto the surface of Cargocytes™. They also showed that these structures were functional, in other words, that the modified Cargocytes™ detected and responded to chemokine guidance signals, adhered to diseased blood vessels, and moved out of the vascular system into the diseased tissue

These experiments were first attempted in Petri dishes but encouraged by their early success, Professor Klemke and the team then conducted experiments in mice. Here, one ear of the animals was deliberately irritated, causing a mild inflammation. When unmodified Cargocytes™ were injected into these animals, they did not leave the bloodstream. However, Cargocytes™ modified with the right chemokine receptors and adhesion molecule rapidly accumulated in the inflamed ear, while practically none were seen in the healthy normal ear on the other side or other organs in the body. Guiding Cargocytes™ to the right location was now a very real possibility.

Loading the Cargo

Finally, Professor Klemke and his team introduced a third modification in which they inserted a purified mRNA that made the Cargocytes™ produce IL-10, a strong anti-inflammatory molecule. When these triple-modified Cargocytes™ were injected into mice with one inflamed ear, their homing to the site of the inflammation worked as expected.

Furthermore, due to the action of IL-10, the swelling of the inflamed ear resolved rapidly. An even more rigorous preclinical test of the triple-modified Cargocytes™ involved tests in animals with inflammation of the pancreas. Sitting deep behind the stomach and hence difficult to approach even during surgery, the pancreas produces digestive juices and insulin. The inflammation of this vital organ is a common life-threatening problem in humans. Experiments demonstrated that triple-modified Cargocytes™ found their way to the damaged gland and the anti-inflammatory effect of IL-10 significantly reduced further damage to the pancreas in the treated mice. Furthermore, the team did not find any evidence of adverse effects associated with the administration of Cargocytes™, instilling further optimism.

Not Perfect – Yet…

These results are extremely encouraging. They show that Cargocytes™ represent a novel and highly promising way to selectively deliver drugs to specific locations. This possibility will open up important new therapeutic approaches although challenges remain. First of all, production and manufacturing methods need to be scaled for clinical applications and the safety of Cargocytes™ needs to be rigorously tested before clinical use. Professor Klemke's team will also need to show that by changing the chemokine receptors and adhesion molecules, they can direct Cargocytes™ to other locations, for example, to a tumour.

This dedicated work means that Professor Klemke is well on the way to making science fiction a reality. In the not-too-distant future, these tiny little biological structures may be able to improve the medical treatment of millions of people.

Meet the researcher

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Professor Richard Klemke earned his PhD in Cell and Developmental Biology from Texas Tech University Health Sciences Center in the USA. After working as a research scientist at Scripps Research (previously known as The Scripps Research Institute), he joined the University of California San Diego's Moores Cancer Center as Professor of Pathology in 2006, where he is now Professor of Pathology and Cancer Biology. Over the past 20 years, Professor Klemke has been a highly active teacher, mentor and lecturer in the academic realm, and also a valued consultant in the biotech and pharmaceutical industries. His laboratory has received numerous federal and private awards to support his development of novel therapeutics for cancer and investigations into the critical mechanisms that drive cancer progression and cancer immunity. Unsurprisingly, Professor Klemke's pioneering work has attracted international recognition, and he continues to provide new insights into the underlying processes of how malignant tumours develop, induce a blood supply, and avoid immune recognition.

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FURTHER READING

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