Scientists design new delivery device for gene therapy

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Scientists have designed a nanoparticle that appears to effectively deliver genetic material into cells with minimal toxic effects.

In lab experiments, the researchers have found that this device, a vector, is able to deliver DNA deeply enough into a cell to allow genetic material to be activated - a critical step in gene therapy. This vector is between 2 1/2 and 10 times more effective than other experimental materials, according to the research.

Biomedical researchers continue to pursue gene therapy as a treatment option for a variety of diseases known to be caused by a genetic defect. That pursuit includes efforts to ensure the safety of the therapy and find the most effective way to deliver the genes.

In many experiments, deactivated viruses that retain their ability to infect other cells are used as vectors to deliver normal genes intended to replace, or turn off, defective genes. But because some of the viruses can generate an immune response that complicates the treatment, scientists also are pursuing nonviral vector techniques for gene therapy.

In this case, Ohio State University scientists combined two ingredients - calcium phosphate and a lipid shell - to create a nanoparticle that protects DNA during its journey to the cell and then dissolves to allow for gene activation in the target cell. Nano refers to the tiny size of the particle in question - its general structure can be detected only by an atomic force microscope.

Calcium phosphate is a mineral found in bones and teeth. Lipids are fatty molecules that help maintain the structure of cell membranes. Alone, calcium phosphate is toxic and lipids get absorbed by cells. Together, they form a protective and inflexible structure that, thanks to complex chemical reactions, self-destructs once inside a cell.

"Our nanoparticle is a foreign body just like a viral vector is, but it has a self-destructive mechanism so it does not generate a strong response from the immune system," said Chenguang Zhou, a graduate student in pharmaceutics at Ohio State and lead author of the study. "The material we use is also biocompatible. Calcium phosphate is in our bones and the lipids we use are synthetic, but can be biologically degraded. That's why there is low toxicity."

The research is published in a recent issue of the *International Journal of Pharmaceutics*.

Zhou noted that other researchers have tried to use liposomes - nanometer-sized bubbles made out of the same material as a cell membrane - to create nonviral vectors for gene delivery. While the material did a good job of protecting the DNA, it did not do a good job of releasing the gene into a cell.

"The liposome gets internalized into cells. It's sort of like eating food that gets stuck in the stomach or intestines, but never gets to the rest of the body," he said.

Similarly, calcium phosphate alone has been considered as a gene delivery vehicle. But because of its salty properties, it becomes unstable and expands in size, which makes it too big to penetrate some cell and vascular walls, and which can cause the immune system to reject it.

"So what we do is encapsulate a calcium phosphate core inside the

liposome," Zhou said. "And when this calcium phosphate gets inside a cell and that environment becomes acidic, it gets dissolved and then the gene can be very effectively released into the cytoplasm and transported to the nucleus. That is the theory."

Zhou and colleagues have developed what they consider an easy method to manufacture this particle. They create a synthetic lipid and place it in a solution that contains calcium and phosphate, which becomes integrated with the lipid. As the acidic properties in the solution change, the calcium phosphate forms a core.

The scientists then mix a solution containing plasmid DNA with their newly formed particle, and all the materials become bundled together. Plasmid DNA is a circular DNA molecule that is able to turn on gene activity that starts a protein-building process without altering an entire genome, or the complete hereditary information of an organism.

Because this particular vector is intended for injection into the bloodstream as a cancer treatment, the particle is designed to protect the DNA from being digested by enzymes as it travels to target cells. A test that exposed this solution to serum, a component of blood, showed that the hybrid particle provided this protection, while unprotected DNA was digested by enzymes.

The researchers next applied this DNA-infused particle solution to mouse cells. The DNA contained the gene code for green fluorescent protein that would be turned on only after it entered the cell. They observed that the particle penetrated the cell membrane and, after a series of interactions occurred, the green fluorescent protein lit up inside the cell, indicating the DNA had reached its target.

For comparison, the group also monitored DNA movement on its own and in other types of vectors. Their hybrid vector was 24 times more

effective at delivering genetic material to the cell than was DNA on its own, and 10 times more effective than calcium phosphate preparations.

"We know the particle gets to where it needs to go and what happens to the particle," Zhou said. "Do we know that the DNA reaches the nucleus? That is something we still need to find out. But because we saw the green fluorescent protein expressed, we think it got to the nucleus or at least as far as the cytoplasm. What's important is that the protein got inside the cell."

The study also showed that this hybrid particle maintained its structure for at least 21 days and, when compared with a variety of other potential vector substances, did very little damage to cells, meaning it is not as toxic as most other materials.

With viral vectors, gene therapy is considered a one-time treatment because when the virus carrying new genes infects a cell, that interaction changes the recipient's entire genome, effectively canceling the activity of the defective gene. Zhou said that with this nonviral vector, treatment would be designed as an intravenous injection on a regular basis until cells are "infected enough to make a change."

The researchers next plan to test the particle's ability to travel through the bloodstream and enter target cells in animals.

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