Tiny mineral particles are better vehicles for promising gene therapy

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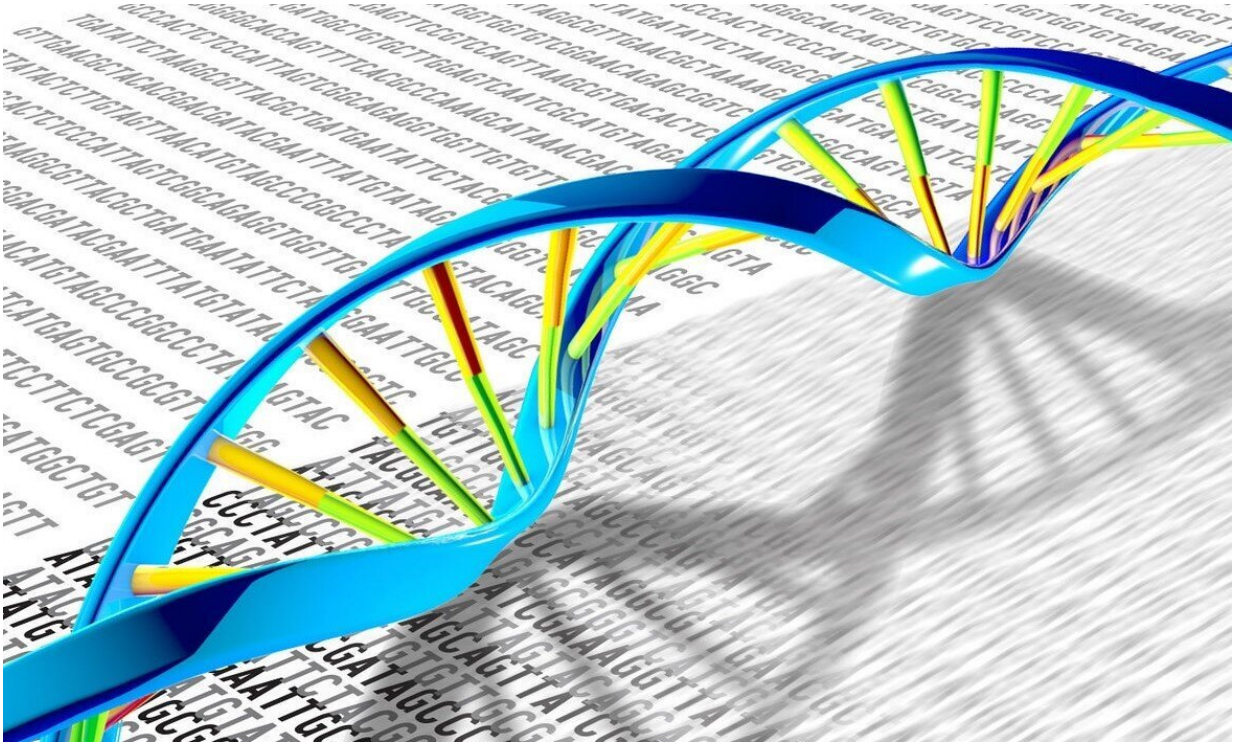

DNA, which has a double-helix structure, can have many genetic mutations and variations. Credit: NIH

University of Wisconsin–Madison researchers have developed a safer and more efficient way to deliver a promising new method for treating cancer and liver disorders and for vaccination—including a COVID-19 vaccine from Moderna Therapeutics that has advanced to clinical trials

with humans.

The technology relies on inserting into cells pieces of carefully designed messenger RNA (mRNA), a strip of genetic material that human cells typically transcribe from a person's DNA in order to make useful proteins and go about their business. Problems delivering mRNA safely and intact without running afoul of the immune system have held back mRNA-based therapy, but UW–Madison researchers are making tiny balls of minerals that appear to do the trick in mice.

"These microparticles have pores on their surface that are on the nanometer scale that allow them to pick up and carry molecules like proteins or messenger RNA," says William Murphy, a UW–Madison professor of biomedical engineering and orthopedics. "They mimic something commonly seen in archaeology, when we find intact protein or DNA on a bone sample or an eggshell from thousands of years ago. The mineral components helped to stabilize those molecules for all that time."

Murphy and UW–Madison collaborators used the mineral-coated microparticles (MCMs)—which are 5 to 10 micrometers in diameter, about the size of a human cell—in a series of experiments to deliver mRNA to cells surrounding wounds in diabetic mice. Wounds healed faster in MCM-treated mice, and cells in related experiments showed much more efficient pickup of the mRNA molecules than other delivery methods.

The researchers described their findings today in the journal *Science Advances*.

In a healthy cell, DNA is transcribed into mRNA, and mRNA serves as the instructions the cell's machinery uses to make proteins. A strip of mRNA created in a lab can be substituted into the process to tell a cell to

make something new. If that something is a certain kind of antigen, a molecule that alerts the immune system to the presence of a potentially harmful virus, the mRNA has done the job of a vaccine.

The UW–Madison researchers coded mRNA with instructions directing cell ribosomes to pump out a growth factor, a protein that prompts healing processes that are otherwise slow to unfold or nonexistent in the diabetic mice (and many severely diabetic people).

mRNA is short-lived in the body, though, so to deliver enough to cells typically means administering large and frequent doses in which the mRNA strands are carried by containers made of molecules called cationic polymers.

"Oftentimes the cationic component is toxic. The more mRNA you deliver, the more therapeutic effect you get, but the more likely it is that you're going to see toxic effect, too. So, it's a trade-off," Murphy says. What we found is when we deliver from the MCMs, we don't see that toxicity. And because MCM delivery protects the mRNA from degrading, you can get more mRNA where you want it while mitigating the toxic effects."

The new study also paired mRNA with an immune-system-inhibiting protein, to make sure the target cells didn't pick the mRNA out as a foreign object and destroy or eject it.

Successful mRNA delivery usually keeps a cell working on new instructions for about 24 hours, and the molecules they produce disperse throughout the body. That's enough for vaccines and the antigens they produce. To keep lengthy processes like growing replacement tissue to heal skin or organs, the proteins or growth factors produced by the cells need to hang around for much longer.

"What we've seen with the MCMs is, once the cells take up the mRNA and start making protein, that protein will bind right back within the MCM particle," Murphy says. "Then it gets released over the course of weeks. We're basically taking something that would normally last maybe hours or even a day, and we're making it last for a long time."

And because the MCMs are large enough that they don't enter the bloodstream and float away, they stay right where they are needed to keep releasing helpful therapy. In the mice, that therapeutic activity kept going for more than 20 days.

"They are made of minerals similar to tooth enamel and bone, but designed to be reabsorbed by the body when they're not useful anymore," says Murphy, whose work is supported by the Environmental Protection Agency, the National Institutes of Health and the National Science Foundation and a donation from UW–Madison alums Michael and Mary Sue Shannon.

"We can control their lifespan by adjusting the way they're made, so they dissolve harmlessly when we want."

The technology behind the microparticles was patented with the help of the Wisconsin Alumni Research Foundation and is licensed to Dianomi Therapeutics, a company Murphy co-founded.

The researchers are now working on growing bone and cartilage and repairing spinal cord injuries with mRNA delivered by MCMs.

More information: Andrew S. Khalil et al. Single-dose mRNA therapy via biomaterial-mediated sequestration of overexpressed proteins, *Science Advances* (2020). DOI: 10.1126/sciadv.aba2422

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