Biologically Inspired: A New Class of DNA-Responsive Materials
by Timothy Erick | September 2019

Deoxyribonucleic acid (DNA) is the alphabet of life. Every living organism has a DNA genome, which serves as an instruction manual for building and maintaining that organism. In the 1950s and 1960s, scientists discovered the structure of DNA and how it encodes biological information. These discoveries inspired the development of techniques for modifying the DNA sequence. Scientists have used these techniques to learn more about genetics and to create genetically modified organisms with useful traits. Recently, scientists have found additional, creative uses for DNA as a substance detached from living organisms.
DNA is remarkably stable, and forms predictable structures based on its sequence. For years, researchers have been incorporating DNA into materials called hydrogels, networks of linked molecules that can absorb a lot of water. DNA hydrogels lend themselves to such applications as drug delivery systems and medical diagnostic devices. Recently, a group of researchers from Harvard University in Cambridge, Massachusetts, and the Massachusetts Institute of Technology, also in Cambridge, used a genome editing technique called CRISPR-Cas12a to make a versatile DNA hydrogel that undergoes structural changes in response to different inputs. Their findings were published in Science, with James J. Collins as the corresponding author.

**Bacterial Immune System**

At first glance, bacteria and viruses might seem similar. They are both microscopic and can cause disease in humans. However, bacteria and viruses are very different. Bacteria are single-celled living organisms. Each bacterium has a DNA genome and a full complement of molecular machinery to carry out biological functions. Viruses, on the other hand, are not considered living organisms. They consist of little more than a genome wrapped in some proteins. Viruses are parasites that can replicate only inside the cells of living organisms, ranging from bacteria to humans.

The human immune system combats viruses by killing infected cells. That works fine for us, since our bodies contain trillions of cells. However, since each bacterium is an independent organism, bacteria need to deal with viruses differently. Bacteria have their own antiviral defense system, called CRISPR-Cas. CRISPR stands for “clustered regularly interspaced short palindromic repeats,” which are repetitive DNA sequences within the bacterial genome. Each of these DNA sequences corresponds to the genome of a different virus. If one of these viruses tries to invade a bacterium, the matching CRISPR sequence is expressed as an RNA molecule, along with a Cas protein. Cas proteins cut nucleic acids (including DNA and RNA) like a pair of molecular scissors. CRISPR RNA guides the Cas protein to the invading virus. The Cas protein then destroys the viral DNA or RNA before it gets a chance to replicate within the bacterium.

Over the past decade, scientists have adapted the CRISPR-Cas system as a genome-editing tool. This system uses a protein called Cas9 along with guide RNA molecules that correspond to specific locations within the genome of target organisms. Using CRISPR-Cas9, scientists can do DNA cutting at particular locations within the genome of almost any organism. Since learning how to use Cas9, scientists have also begun using a related protein, called Cas12a. Much like Cas9, Cas12a cuts target double-stranded DNA (dsDNA) that corresponds to a particular guide RNA. However, while Cas9 makes only one cut, Cas12a proceeds to rapidly and indiscriminately cut up single-stranded DNA (ssDNA). After initially cutting its target dsDNA sequence, Cas12a can degrade ssDNA at the rate of roughly 1,250 cuts per second.
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Slicing and Dicing

The indiscriminate ssDNA cutting activity of Cas12a caught the attention of Collins, a researcher at Harvard's Wyss Institute for Biologically Inspired Engineering. Collins has long been interested in combining biology with engineering to create designer organisms with useful features. Collins and his team of researchers realized that CRISPR-Cas12a could perhaps be used to design DNA hydrogels for a variety of applications.

Hydrogels are solid materials that consist of networks of linked molecules called polymers. Hydrogels have unique properties based on their structure and molecular makeup. Scientists can attach individual DNA building blocks to the polymers used to make hydrogels. This allows for the creation of DNA hydrogels that not only are compatible with living organisms but can also be structurally modified to deliver drugs or detect pathogens.

Researchers at Harvard University and the Massachusetts Institute of Technology, both in Cambridge, Massachusetts, created hydrogels in which some of the polymers were linked to ssDNA sequences. When they introduced Cas12a, a guide RNA, and the corresponding target dsDNA molecule into the hydrogel, Cas12a cut the dsDNA. This initiated a chain reaction in which Cas12a cut all of the ssDNA molecules within the hydrogel, breaking the links between polymers.
Collins and his team took this idea one step further by creating hydrogels in which some of the polymers were linked to ssDNA sequences. When they introduced Cas12a, a guide RNA, and the corresponding target dsDNA molecule into the hydrogel, Cas12a cut the dsDNA. This initiated a chain reaction in which Cas12a cut all of the ssDNA molecules within the hydrogel, breaking the links between polymers. The researchers tested this system by making a hydrogel in which fluorescent molecules were attached to the polymers with ssDNA. After they introduced Cas12a, guide RNA and target dsDNA into the hydrogel, the ssDNA linkers were degraded and the fluorescent molecules were released from the hydrogel into the solution. Cas12a and the guide RNA did not release the fluorescent molecule unless and until the dsDNA trigger was introduced, suggesting that this system could be easily controlled.

Swiss Army DNA Scissors

The researchers tested several possible applications for their programmable hydrogel. First, they anchored gold nanoparticles within a hydrogel using ssDNA linkers. Introduction of Cas12a, guide RNA and the appropriate dsDNA destroyed the linkers, releasing gold nanoparticles into the surrounding solution. The researchers also used ssDNA to encapsulate human immune cells into a hydrogel. Much like in their previous test, introduction of Cas12a, guide RNA and dsDNA caused the release of the immune cells. In both cases, the cargo was released from the hydrogel only after Cas12a had been combined with the appropriate guide RNA and dsDNA.

Casting an even wider net, the researchers then constructed a hydrogel consisting of electrically conductive nanoparticles linked to ssDNA molecules. This new hydrogel was used to seal off the open end of a silver electrode, creating a closed circuit capable of conducting electricity. The researchers immersed the electrode-hydrogel circuits into a conductive solution, and then added Cas12a, guide RNA and the corresponding dsDNA. The cutting action of Cas12a caused the degradation and detachment of the hydrogel from the electrode, breaking the circuit and halting the flow of electricity. The team had in effect created a biological fuse capable of responding to dsDNA inputs.

Finally, the researchers constructed a medical device for detecting viruses. It consisted of multiple layers of filter paper with an ssDNA hydrogel inserted
between the layers. A liquid buffer containing a human blood sample, Cas12a and guide RNA corresponding to a particular virus was added to the top layer, and an electronic circuit designed to monitor buffer flow was inserted in the bottom layer. The intact ssDNA hydrogel was designed to stop the buffer from flowing through the layers. If viral DNA was present in the human sample, the Cas12a-guide RNA complex would degrade the ssDNA hydrogel. This would allow the buffer to flow all the way through to the final chamber, where it would be detected by the circuit. The researchers found that the system could detect minute concentrations of viral dsDNA. Some viruses have RNA genomes, so the researchers made a modified version of the device that contained reverse transcriptase, a protein that converts RNA to dsDNA. This version was able to detect the RNA genome of *Ebolavirus*. The researchers thus appear to have created a prototype for a simple and inexpensive viral diagnostic system that could be used almost anywhere.

This research may eventually serve as the basis for applications ranging from timed release of medications to construction of new tissues and organs to improved detection of a variety of pathogens. With the level of control afforded by CRISPR-Cas12a, the sky may well be the limit.

**Discussion Questions**

What are the advantages and disadvantages of Cas12a compared to its predecessor, Cas9?

Can you think of situations where scientists might want to continue to use Cas9 rather than switch to Cas12a?

**Journal Abstracts and Articles**

*(Researchers own descriptions of their work, summary or full-text, on scientific journal websites.)*


**Bibliography**


**Keywords**

DNA, nucleic acid, CRISPR, Cas9, Cas12a, guide RNA, double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), hydrogel, polymer, Ebolavirus, James J. Collins

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**Record URL:** https://tsof.infobaselearning.com/recordurl.aspx?wid=10835&ID=43297

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