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March 18, 2014

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By Andrea Anderson

University of Ottawa researchers have developed an electrical method for fabricating solid-state nanopores based on a phenomenon called dielectric breakdown — an approach that they are touting as fast, affordable, and easily applicable within the context of existing solid-state detection systems.

"What we feel about this method is that it's going to democratize the field," the University of Ottawa's Vincent Tabard-Cossa told *In Sequence*.

"There's no more cost associated with fabricating a pore," he said. "You've got your membrane and that's the most expensive part. The rest of the circuit is the same as you'd use for doing experiments."

Tabard-Cossa is senior author on a pair of papers describing the approach and its validation. The first of these appeared in the online preprint study archive ArXiv this past October. An accompanying study published in *Small* early this month demonstrated that members of the team could reliably use the approach to produce nanopores with 2 nanometer diameters in nearly two-dozen consecutive experiments.

With that pore diameter, the researchers noted, it becomes possible to pass individual strands of single- or double-stranded DNA through the pore single file, though they are not currently using the dielectric breakdown drilling method to do DNA sequencing.

"We're not describing a sequencer with this technique yet," Tabard-Cossa said, noting that the solid-state nanopore sequencing approaches described so far typically rely on very thin membranes — often comprised of graphene — in order to register each nucleotide as a given DNA molecule moves through the pore.

At the moment, the group is using its dielectric breakdown approach to drill nanopores into relatively thick membranes made of other materials, though the same general method is expected to be applicable to graphene.

Even so, Tabard-Cossa noted that there are other research and clinical applications that can be done using nanopores that do not require fully sequencing a DNA molecule, including targeted mutation or sequence detection.

Should the dielectric breakdown approach make its way into a sequencing instrument in the future, though, he envisions a system in which nanopores are drilled into a system in solution immediately prior to translocating and detecting DNA.

"The pore would be fabricated the instant the user needed the pore," Tabard-Cossa explained.

"There [would be] no need to create a pore, do in-house quality control before shipping it, and making sure it doesn't expire," he added. "There's a simplicity in this. All you'd need is a packaged membrane."

When he took the helm of his own lab at the University of Ottawa several years ago, Tabard-Cossa planned a research program that involved using solid-state molecules to study confined geometries, including that of DNA molecules with an eye towards doing sequencing and other detection applications with such nanopores.

When those efforts began, he and his team relied on transmission electron microscope, or TEM, technology to drill pores in membrane material by focusing a beam of electrons using a transmission electron microscope.

Although almost every research group doing solid-state nanopore-related research uses TEM, Tabard-Cossa explained, the method is time-consuming. And in his lab, students found it tricky to apply TEM to consistently produce stable pores of the appropriate size.

"That's been a big hurdle for the field," he said. "A lot of people who try to get into the field have a hard time making the pores — or making them behave the way they want them."

He and his colleagues came up with a completely different approach to drilling nanopores using custom electronics set up in the lab.

While these setups roughly resembled the well-established systems used for translocating DNA through solid-state nanopores, they included current amplifiers capable of generating up to 10 or 20 volts rather than the typical 1 volt output of the Axopatch — the current amplifier normally used to move DNA through solid-state nanopores and to record the ionic current signal produced during this process.

Initially, the researchers used that system to enlarge the diameter of existing solid-state nanopores — an approach they described in the journal *Nanotechnology* in 2012 and later elaborated on in a video that appeared in the *Journal of Visualized Experiments*.

"We showed that we could continuously grow a nanopore to another size just by applying higher voltages across it," Tabard-Cossa said.

Following the first successful attempt to modify existing solid-state nanopores by applying higher voltages across them, one of Tabard-Cossa's students, Harold Kwok, went a step further, applying voltage to an undrilled membrane in the same system.

Kwok placed a 30 nanometer-thick membrane in the system continuously for a couple days and exposed it to around 10 volts, following the signal as current passed through the insulating material. He found that "there was actually current passing through the membrane, even though it's an insulator," Tabard-Cossa explained. "It's some kind of tunneling current."

The serendipitous experiment produced a fairly large pore, on the order of a few hundred nanometers in diameter.

In the process of unraveling the reason for that pore formation, the researchers realized they could use a similar approach to create carefully controlled pores in various materials, including silicon nitride and silicon dioxide.

"We repeated the experiments in different conditions and looked more closely at the 'leakage' current that we saw passing through the insulating membrane," Tabard-Cossa said.

"What we saw was that at some instant there was a gigantic spike in the current. There was an event where the current was stable and then it exponentially increased very rapidly," he noted. "We thought that that resembled, very much, what people in the semiconductor field call a dielectric breakdown event."

Such events are analogous to a lightning strike, which involves charges piling up in clouds and on earth, eventually breaking down the insulating air so that current can span the space between.

Tabard-Cossa noted that the dielectric breakdown process has been largely studied in the context of semiconductor failure, which causes semiconductors to start conducting electricity under high electric field stress.

Following the initial demonstration that the dielectric breakdown could produce pore-like holes in a membrane, the researchers determined that they could control the size of such pores by carefully monitoring the system during the process.

When the researchers tried creating pores under controlled conditions, they found that they were getting pores in the 5 to 10 nanometer diameter range simply by following current feedback after applying voltage to the system.

Moreover, by increasing the electric field strength, the team is now harnessing dielectric breakdown to make pores in as little as a few seconds. That raises the possibility of producing pores on the fly right before using them in experiments — something members of Tabard-Cossa's lab are already doing.

By measuring pore conductance, the team is able to accurately estimate pore sizes as they form assuming the pore geometry remains the same, with a cylindrical pore.

"We stop it as soon as we see one breakdown event," Tabard-Cossa said. "And that allows us to make a single pore in the membrane."

With additional tweaking to the method, for example, the researchers showed that it was possible to produce even smaller pores, on the order of around 2 nanometers apiece. As they reported in *Small*, for example, the researchers could reproducibly use dielectric breakdown to drill 2 nanometer pores in silicon nitride membranes.

The resulting pores were larger than single-stranded DNA, but too small for double-stranded molecules to pass through unhindered, allowing the team to use DNA itself to measure pore size.

"We used DNA as a molecular ruler because we know DNA geometry, we know the length, we know the diameter, and we can use that to actually size the pore," Tabard-Cossa said.

In that study, the researchers described having 100 percent success creating 2 nanometer pores in 10 nanometer thick silicon nitride membranes over 23 consecutive experiments.

They also showed that it was possible to use the resulting pores as makeshift tweezers for biophysics experiments that involved stretching and scrutinizing bits of double-stranded DNA trapped inside the pores.

Generally speaking, the dielectric breakdown method appears to be applicable both in solution, at a range of pHs, in non-aqueous conditions, and using several different membrane materials, Tabard-Cossa said.

The precise mechanism by which material gets removed from a membrane during these breakdown events is still somewhat murky, he noted. "One thing we don't really know for sure is the mechanism for material removal. We're not really sure what happens, but we make really nice holes."

Although nanopore formation through dielectric breakdown remains a stochastic process, Tabard-Cossa noted that there are relatively simple ways to influence where the pore will form in a given membrane.

In preliminary, unpublished work, the researchers have been looking at ways of using the dielectric breakdown process to produce arrays by using microfluidic channels to separate portions of the same membrane, making it possible to electrically address different parts of the membrane independently.

For their part, he and his team are applying dielectric breakdown to produce pores as part of several different projects, including efforts aimed at developing clinical diagnostic applications based on finding particular DNA sequence mutations with the help of probes and double-stranded DNA unzipping with nanopores.

"We're not yet designing a detector to detect the bases, we're just making the holes so far," Tabard-Cossa said. "But I think the technology could be translated to many of the other approaches that people are using to read DNA."

The University of Ottawa has filed for multiple patents related to the use of dielectric breakdown both for creating and enlarging nanopores. Tabard-Cossa said that he has discussed the approach with commercial entities, but did not disclose the identity of potential collaborating companies.

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