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Tracking lab-grown tissue with light

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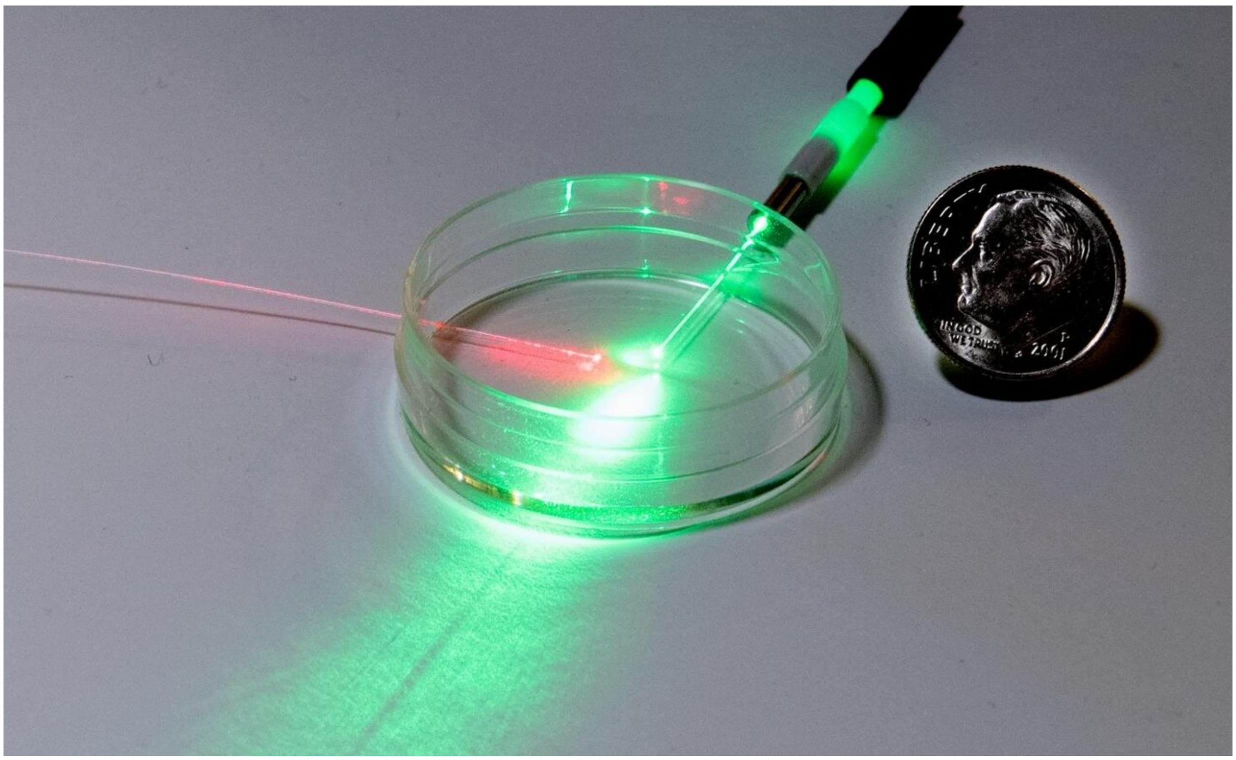

An empty petri dish with two optical fibers, illustrating one version of the researchers' experiment. The left-hand fiber (usually shining infrared light, but depicted here as visible red light) is a temperature sensor. The top fiber shines green, red or blue light into the petri dish to adjust the signal that the temperature sensor measures. Credit: J.L. Lee/NIST

Someday, doctors would like to grow limbs and other body tissue for soldiers who have lost arms in battle, children who need a new heart or liver, and many other people with critical needs. Today, medical professionals can graft cells from a patient, deposit them onto a tissue scaffold, and insert the scaffold into the body to encourage the growth of bone, cartilage and other specialized tissue. But researchers are still working toward building complex organs that can be implanted into patients.

Scientists at the National Institute of Standards and Technology (NIST) are supporting this field of research by developing a promising new kind of light-based sensor to study tissue growth in the lab.

The NIST team's proof-of-concept work, published today in *Sensors and Actuators B*, demonstrates a small sensor that uses a light-based signal to measure pH, the measurement unit for acidity, an important property in cell-growth studies. The same basic design could be used to measure other qualities such as the presence of calcium, cell growth factor and certain antibodies.

Unlike conventional sensors, this measurement method could be used to monitor the environment in a cell culture long-term—for weeks at a time—without having to disturb the cells regularly to calibrate the sensing instruments. Watching properties of the tissue in real time as they slowly change, over days or weeks, could greatly benefit tissue engineering studies to grow teeth, heart tissue, bone tissue and more, said NIST chemist Zeeshan Ahmed.

"We want to make sensors that can be put inside growing tissue to give researchers quantitative information," Ahmed said. "Is the tissue actually growing? Is it healthy? If you grow a bone, does it have the right mechanical properties or is it too weak to support a body?"

The work could have benefits beyond tissue engineering too, into studying the progression of diseases such as cancer.

"What these sensors could give people is real-time information about tissue growth and disease progression," said American University chemist and NIST guest researcher Matthew Hartings. Conventional sensors give researchers a series of snapshots without showing them the path between those points, Hartings said. But photonic sensors could provide scientists with continuous information, the equivalent of a GPS navigation app for disease.

"We want to provide researchers with a detailed map of the incremental changes that happen as tissue either grows in a healthy way or becomes diseased," Hartings said. "Once researchers know the 'streets' a disease is taking, then they can better prevent or support the changes that are happening" in a patient's body.

A Problem to Solve

Measurements of pH are a vital part of tissue engineering studies. As cells grow, their environment naturally becomes more acidic. If the environment becomes too acidic—or too basic—the cells will die. Scientists measure pH on a scale from 0 (very acidic) to 14 (very basic), with an ideal environment for most cells in a narrow range around a pH of 7.

Commercial pH instruments are highly accurate but unstable, meaning they require frequent calibrations to ensure accurate readings day to day. Without calibration, these conventional pH meters lose up to 0.1 pH units of accuracy daily. But tissue engineering studies take place on the order of weeks. A culture of stem cells might need to be grown for almost a month before they turn into bone.

"An increment of 0.1 pH is significant," Ahmed said. "If your pH value changes by 1, you kill the cells. If after a few days I can't trust anything about my pH measurement, then I'm not going to use that measurement method."

On the other hand, if researchers disturb the growing cells every time they have to measure the cell culture's pH, then the scientists are introducing another kind of uncertainty to their measurements, since they are altering the cells' environment.

What's needed for this kind of research, Ahmed said, is a measurement system that can stay inside an incubator with the cells in their culture medium and not need to be removed or calibrated for weeks at a time.

Brave New Sensors

For years, Ahmed and his team have been developing photonic sensors, small lightweight devices that use optical signals to measure a range of qualities including temperature, pressure and humidity.

Some of these novel devices use commercially available, flexible optical fibers etched with a Bragg grating, a kind of filter for light that reflects certain wavelengths and allows others to pass. Changes in temperature or pressure alter the wavelengths of light that can pass through the grating.

To adapt their photonic devices to a pH measurement, Ahmed and Hartings relied on a well-known concept in science: When an object absorbs light, the energy absorbed "has to go somewhere," Ahmed said, and in many cases that energy turns into heat.

"For every individual photon, the heat produced is a very small amount of energy," Ahmed said. "But if you have lots of photons coming in, and you have lots of molecules, it becomes an appreciable change in heat."

For their demonstration, the scientists used a substance that changes color in response to changes in pH, a material that many people may remember from biology classes: red cabbage juice powder. Cabbage juice changes its color from shades of dark purple to light pink depending on the acidity of a solution. That change in color can be picked up by Ahmed's photonic temperature sensors.

Researchers filled a petri dish with the cabbage juice solution. One optical fiber was positioned above the dish. It was connected to a laser pointer and shined light into the sample. A second optical fiber was physically embedded in the liquid. This second fiber contained the Bragg grating and acted as the temperature sensor. Ahmed's team controlled the solution's pH manually.

To make a measurement, the researchers shone one color of light—such as red—into the sample from above. The cabbage juice absorbed the red light to varying degrees based on its color, which depended on the pH of the solution at that time. The photonic thermometer fiber picked up these slight changes in the juice's heat. A change in temperature changes the wavelengths of light that can pass through the fiber's Bragg grating.

Next, the researchers shone a second color of light—such as green—into the liquid, and repeated the process.

By comparing how much heat was generated by each color of light, researchers could determine the exact color of the cabbage juice at that moment, and that told them the pH.

"Literally we said, 'Can we turn two laser pointers on and off for a few minutes and see if we can turn that into a pH meter?'," Ahmed said. "And we were able to show that it works over a wide range," from a pH of 4 to a pH of 9 or 10.

Ongoing work shows the photonic pH measurements are accurate to plus or minus 0.13 pH units and are stable for at least three weeks, much longer than conventional measurements.

Beyond Cabbage Juice

The researchers say that according to their tissue engineering collaborators, the new photonic sensors could provide useful information for a range of biological systems being studied, particularly the growth of heart and bone cells.

For their next round of experiments, already underway, the NIST researchers are using another pH-sensitive dye called phenol red. In addition, they are working to encapsulate the dye in a plastic coating around the fiber itself so that it does not interact with the cell medium. The team is also conducting its first test of the system in a real cell culture, with help from NIST colleagues who specialize in tissue engineering.

Future plans include measuring quantities beyond pH, which would simply require swapping out phenol red for a different dye sensitive to whatever property researchers want to measure.

And much further in the future, Ahmed hopes the measurement scheme could potentially be used to monitor the growth of tissue in a real person's body.

"The long-term goal is being able to put implantable devices into people where you're trying to grow bones and muscles, and then hopefully over time the sensors could be designed to dissolve away and you wouldn't even have to go back in and remove them," Ahmed said. "That's the ultimate dream. But baby steps first."

More information: Matthew R. Hartings et al, A photonic pH sensor based on photothermal spectroscopy, *Sensors and Actuators B: Chemical*

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