RESEARCH PROFILE

Exploring cell insides by explosion

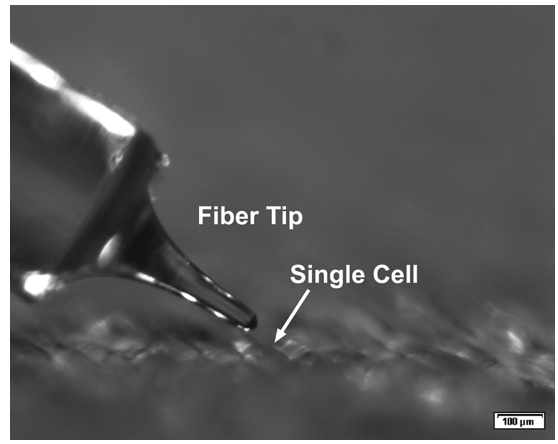
Profiling the contents of a single cell can provide insight into cell metabolism and division, disease states, and environmental impact on biological processes. MS has shown tremendous potential for identifying metabolites in cells, but until now, the only way to interrogate a single cell was to remove it from or change its environment before data collection. But a recent *AC* paper (**2009**, DOI 10.1021/ac901525g) describes a new way to study single cells within a more natural context: by using laser ablation to rapidly vaporize the cytoplasm for subsequent ionization and analysis by MS.

"The cell can still be alive, functioning normally, when we start the experiment," says the study's principal investigator Akos Vertes of George Washington University. "You instantaneously—that is, on the physiological time scale of the plant or animal—blow up a cell, vaporizing its contents and performing the analysis in a fraction of a second."

The paper describes the capture of metabolic profiles from the epidermal cells of two types of plants—the onion and wild daffodil—as well as sea urchin eggs.

"I think it's an important achievement...because they've been able to examine contents of single cells. That's not unique, but what is unique is being able to do that in the ambient environment," says R. Graham Cooks of Purdue University. "If it were my work, I'd be tremendously excited by being able to look at metabolites and other compounds in cells where there is no treatment of the sample and the measurement is direct."

The key to Vertes and coauthor Bindesh Shrestha's technique was to reduce the size of the ablation craters to less than the size of a single cell, allowing them to target one cell at a time. "We used conventional optics before, but there are many factors that keep you from focusing down to the size of a plant cell," says Vertes. So instead, they borrowed an idea from near-field optical microscopy. "In this microscopy, people overcome the diffraction limit by sharpening the optical fiber and bringing it very close to the imaged object," he says.



The sharpened GeO_2-based glass optical fiber, pictured above plant tissue; this fiber was used to deliver laser pulses to single cells.

They sharpened their GeO_2-based glass optical fiber by chemically etching its tip with HNO_3, bringing a 450-μm-diameter core into a sharp tip with a radius of curvature of 15 μm. The tip was mounted onto a micromanipulator and, using a long-distance video microscope, adjusted to maintain a tip-to-sample distance of ~30−40 μm.

A second camera was used to align the optical fiber over a cell. Ablating a plant cell was simply a matter of aiming the sharpened end of the fiber at the plant tissue and firing the mid-IR laser, but to get at the sea urchin egg cell, the researchers needed to first manipulate and immobilize the cell with a holding pipette.

The cytoplasm vapor consisted of mostly neutral species, but as they rose up from the vanquished cell, these chemicals were met by an electrospray plume that converted them to ions for MS. With this setup, the researchers began to observe mass spectra at about the second laser pulse. All cellular contents were ablated after ~100 pulses.

"One complication is that in this article they vaporize the material and then use [ESI]. The combination of the two techniques may yield ionic species or fragments that may not be anticipated [by using either method alone] and may impact identification," says Edward Yeung of Iowa State University. "On the other hand, this could be an opportunity to control ionization in a specific way."

To assist with peak identification, Vertes and Shrestha used MS/MS. "If two molecular ions have the same mass, we can likely still tell them apart based on the tandem MS," says Vertes. "[In this study] we detected >300 peaks and identified >30 of them, but nothing prevents us from identifying all of them."

Although they did arrive at some interesting findings in the plant and sea urchin cells—such as greater flavonoid content in pigmented onion cells than in unpigmented cells and age-related differences—the authors are eager to develop this technique further for the study of mammalian cells.

"In the next stage we are planning to go to ~10 μm, because that is the size of a typical mammalian cell or a white blood cell," says Vertes. "At that point we can really look at metabolic changes due to disease and metabolic factors in more detail."

—Erika Gebel

10.1021/AC9021314 © 2009 AMERICAN CHEMICAL SOCIETY
Published on Web 10/01/2009