Molecular cellophane

MASS SPECTROMETRY

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In vacuum, a monolayer graphene cover enables imaging mass spectrometry of living, wet cells.

Ron M. A. Heeren

Molecular imaging mass spectrometry (MS) has taken another leap into the future. The group of Dae Won Moon at the Daegu Gyeongbuk Institute of Science & Technology in South Korea has developed an innovative method that allows the study of living cells inside the vacuum of an imaging ion source[1]. Their secret: using a graphene layer as molecular cellophane to protect living cells from the harsh vacuum environment (Fig. 1). This is a remarkable achievement. Many will start using imaging MS to study the local dynamics of life without the need for labels — something that has not been possible to date. Lim et al.[1] describe the details of this innovative approach in a paper published in this issue of *Nature Methods*.

Live cell imaging using molecular probes that target specific proteins and optical microscopy is common ground in cellular biology. However, in MS-based molecular imaging, live cell imaging has remained one of the holy grails. The ability to study a wide range of small and larger molecules on cellular membranes, without interfering labels, could offer more precise insight into the regulatory processes of life and their dynamics. Using energetic primary ions to generate secondary ion mass spectrometry (SIMS) images from a biological surface offers a spatial resolution advantage over the use of chemical matrices and laser beams of approximately an order of magnitude.

Several groups have attempted to capitalize on this advantage and used ion-beam-based cellular imaging with subcellular-resolution imaging MS. Whether targeted (using mass labels) or untargeted, almost all studies have been performed on freeze-dried, freeze-fractured, cryoprepared or even cryosectioned cells, frozen in time. As a result, all of these methods rely on dead cells. In addition, it is all too easy to introduce various types of sample preparation artifacts that may influence the outcome of the experiment. New avenues are being explored to break this boundary. Researchers at Pacific Northwest National Laboratory have developed a microfluidic approach to encapsulate a living cell and then use a ion beam to drill a hole through a SiN cover to study the cellular membrane at the liquid–vacuum interface[2]; however, imaging a large surface is challenging with that method.

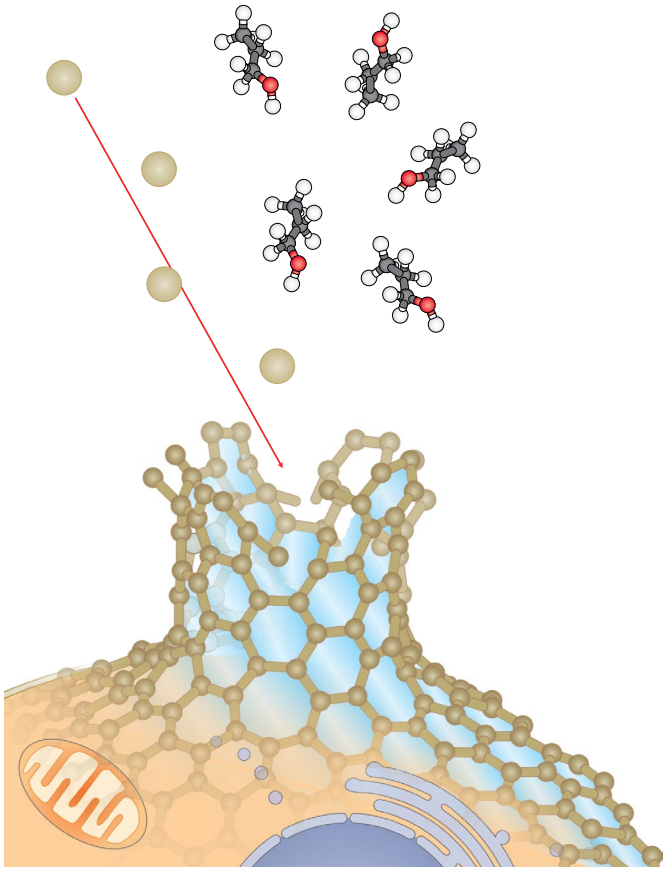

Fig. 1 | An artist's impression of a monolayer of graphene that covers a living, 'wet' cell impacted by primary ions. The secondary, organic ions of membrane cholesterol are ejected through a nanoscopic hole in this molecular-cellophane-like graphene layer and analyzed by a mass spectrometer. This allows direct in-vacuum molecular imaging of living cellular systems.

Lim et al. have taken a radically different approach. Their method uses a graphene layer to cap the cells and retain all cellular water at room temperature, even under vacuum. But can the approach work as described? After all, SIMS sputters material away, so how does the graphene layer stay intact to protect the cells? Are the cells still viable after the imaging experiment? The paper's supplementary video showing cell viability, combined with additional cell viability studies, demonstrates that graphene-covered cells prepared on a wet substrate with a cell culture medium reservoir survived not only the vacuum environment but also repeated static SIMS imaging cycles. The images that show the cholesterol distribution in approximately 50 treated and untreated single living cells demonstrate cellular viability even after 30 min in vacuum.

The quality of the images and the spectral (molecular) content closely resemble those of normal freeze-dried or

cryoprepared cells. This indicates that the ionization mechanism is not compromised (nor enhanced) by the graphene capping. The larger organic molecules can only escape the surface if the holes in the graphene cover induced by the impact of the primary ion are big enough. Their experiments seem to indicate that the edge of the 'hole' reacts with water to form graphene oxide, in a process that resembles regeneration, and one may speculate that the surface 'reseals'. This could also offer time for the living cellular membrane to biologically regenerate, as a static SIMS imaging experiment typically probes less than 1% of the surface. There are many applications for this approach in high-resolution molecular imaging of living systems, from drug–membrane interactions to the study of cellular membrane dynamics.

This does merit the question: what's next? I am convinced that the broader MS imaging community will pick up this method to further our understanding of membrane processes. The chemical dynamics of continuously changing surfaces has been challenging to investigate so far. Graphene capping might provide a way to study wet catalytic surfaces, examine reactions at solvent fronts, or even use molecular imaging to read out metabolic processes in 'organ-on-a-chip' devices. Using functionalized graphene[3] or graphene oxide could target controlled manipulation of cellular molecules or enhance ionization efficiencies for imaging MS. Improved sensitivity will result in better spatial resolution, and graphene has already been shown do to just that[4]. There is much new science that is now within reach. I cannot wait to start to use molecular cellophane and try this in the lab myself to see what we can keep fresh and alive in our imaging mass spectrometers. ❐

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Competing interests

The author declares no competing interests.