Atomic force microscopy reveals high heterogeneity in bacterial membrane vesicles

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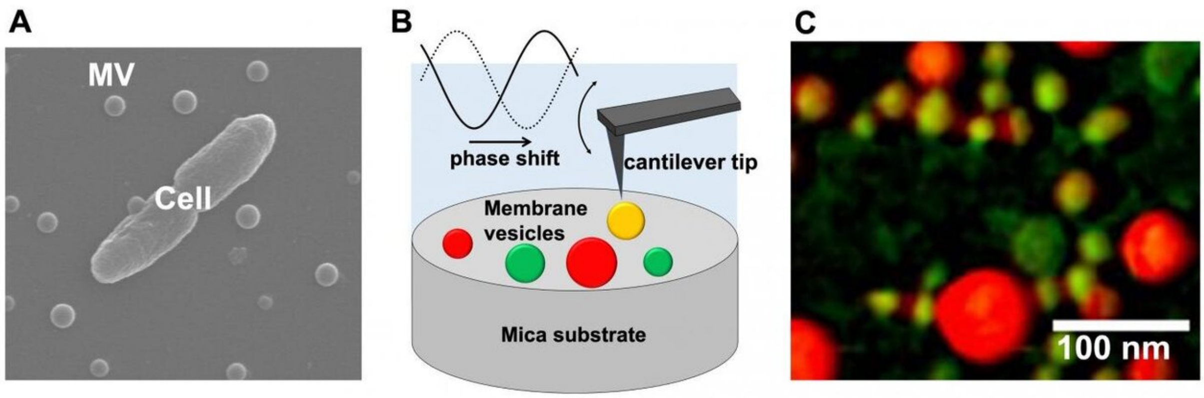

(A) Scanning electron microscopic image of a bacterial cell and extracellular membrane vesicles (MVs). (B) Schematic drawing of MVs observation using atomic force microscopy phase imaging. (C) Mapping of MVs' physical properties using atomic force microscopy phase imaging. MVs are color-coded on a scale ranging from "non-adherent/hard" (reddish-coloured spheres) to "adherent/soft" (greenish-coloured spheres). Credit: Kanazawa University

One aspect of bacterial activity is the production of so-called extracellular membrane vesicles (MVs): biological 'packages' wrapped in a lipid-bilayer membrane, carrying for example genetic material. Apart from having specific biological functions, MVs are increasingly used in nanobiotechnological applications, including drug delivery and enzyme transport. In order to better understand the processes involving MVs, a full apprehension of their physical properties is essential. In particular, the degree of heterogeneity of vesicles released by one single type of bacterium is an important point. Now, Azuma Taoka from Kanazawa University, Nobuhiko Nomura from Tsukuba University and colleagues have addressed this question, and demonstrate a previously unrecognized physical heterogeneity in the membrane vesicles of four types of bacterium.

The researchers applied phase imaging atomic force microscopy (AFM) to study the physical properties of MVs produced by *E. coli, P. aeruginosa, P. denitrificans and B. subtilis*. In phase imaging AFM, a sample is 'tapped' with a nano-sized oscillating cantilever tip; the observed delay in the oscillation of the tip compared to free oscillation provides a measure of the energy dissipation due to the interaction with the sample surface. This dissipation, in turn, is related to the physical properties of the surface, including adhesion, elasticity and friction, variations of which are due to compositional differences.

Taoka, Nomura and colleagues recorded phase images of many MVs, and color-coded the MVs on a scale ranging from "non-adherent/hard" (low adhesion, elasticity and/or friction) to "adherent/soft" (high adhesion, elasticity and/or friction). By analyzing these maps, the scientists discovered a high diversity of physical properties of MVs. They checked whether the maps changed during imaging; the physical properties were stable in time, so the diversity could be concluded to be an intrinsic feature of MVs.

The researchers found that the physical heterogeneity is caused by biological factors, as MV size and phase shifts are not correlated. Furthermore, they observed that different types of bacterium form MVs with different physical-property distributions. Finally, the scientists argued that the observed high heterogeneity reflects the chemical composition of the MVs being heterogeneous.

The work of Taoka, Nomura and colleagues not only presents important insights into the properties of MVs produced by different bacteria, but also shows the power of phase shift AFM as a tool for biological vesicles. Quoting the researchers: "It is expected that using these cutting-edge techniques for nanoscale physical mapping will contribute to provide further detailed information to undiscovered nature of bacterial MVs and elucidate molecular mechanisms supporting their functions."

More information: Yousuke Kikuchi et al, Diversity of physical properties of bacterial extracellular membrane vesicles revealed through atomic force microscopy phase imaging, *Nanoscale* (2020). [DOI: 10.1039/C9NR10850E](https://dx.doi.org/10.1039/C9NR10850E)

Provided by Kanazawa University

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