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Flow cytometry zooms in on extracellular vesicles

Extracellular vesicles can provide a wealth of information, but these intriguing messengers are notoriously difficult to detect. This could be about to change.

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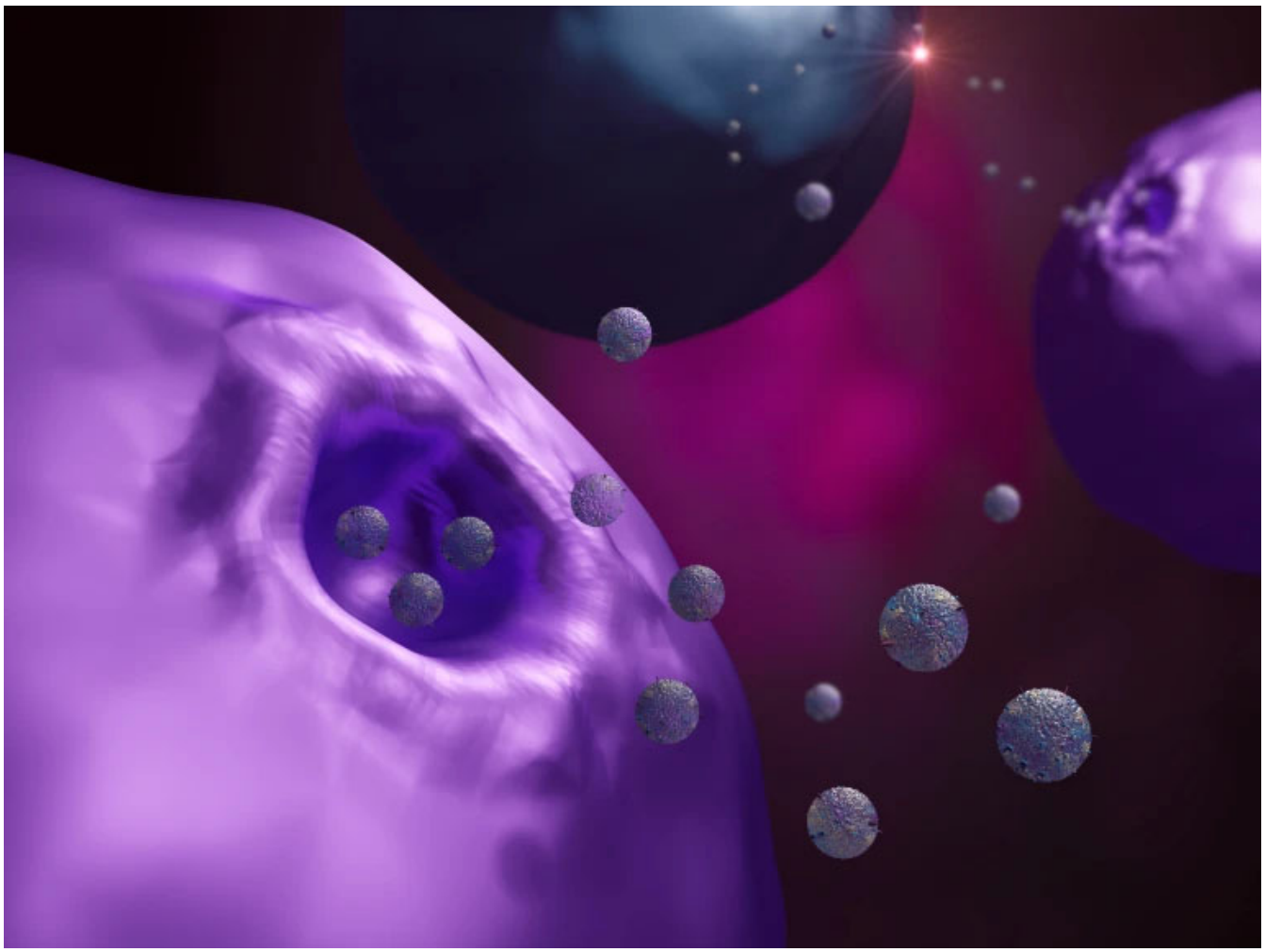

Cells secrete exosomes, a type of extracellular vesicle which can be detected in all body fluids. Credit: Meletios Verras/Shutterstock

Extracellular vesicles (EVs) are a heterogeneous group of subcellular particles containing diverse biological cargo from the releasing mother cell. EVs have a role in cell communication and can be detected in all body fluids. However, their characteristics can change when cells or tissues are damaged or disrupted. Expanding knowledge around these particles could lead to better understanding of a range of health conditions, from cancer to cardiovascular disease — and also to the development of novel EV-based diagnostics and therapeutics.

“If we look closely at EVs, we can find out what's happening, even when it’s too early to see in other ways,” says Jonni Moore, professor of pathology and laboratory medicine at the Hospital of the University of Pennsylvania. It's possible to detect cardiovascular anomalies or even signs of a concussion via plasma (Kumar, Cyto2020 abstract).

Moore has used flow cytometry to create cardiovascular health profiles based on blood samples. However, the challenging nature of EVs, instrument limitations and ambiguity around methods and data interpretation means that it requires specialized flow cytometry technology and standards, which are just becoming available now.

Dim, complex and unpredictable

“The main challenge when using flow cytometry to detect EVs is that the signals are dimmer than cells, complex, and have arbitrary units,” says Edwin van der Pol, assistant professor in biomedical engineering and physics at Amsterdam University Medical Centers. Alongside research into EVs as health markers, he works to solve reproducibility issues in EV detection.

Flow cytometry detects light that scatters off cells, EVs or any other reference particles like polystyrene beads. If all particles had the same shape and composition, the output measurement would be directly comparable. But, van der Pol cautions, “the relation between the size, the composition of a particle and the light scattering signal is complex.” This makes it difficult to directly use standard reference beads for size-based gating and to get reproducible, reliable readings of heterogeneous components like EVs.

In 2020, van der Pol helped to develop an EV-flow cytometry specific framework known as MIFlowCyt-EV: Minimum Information about a Flow Cytometry experiment for extracellular vesicles. Since then, he has been quantifying and reporting the size range and fluorescence range for all his EV measurements. Combined with tools like software and reference beads to calibrate output, such standardized reporting makes it possible to compare EV data measured in different labs. “For the first time, EV flow cytometry measurements become reproducible,” says van der Pol, “and many more labs are doing this now.”

A matter of size

Another challenge in EV detection is that many flow cytometers were optimized to detect cells in the range of 10 to 100 micrometers, but smaller particles, like nanosized EVs, often get lost in the noise and a sea of plasma proteins.

New flow cytometers have been developed to solve these challenges. One such cytometer from BD Biosciences has a small particle detector that can reliably identify and count particles as small as 90 nm over a broad dynamic range of concentrations. According to Moore, this size range includes most clinically relevant EVs.

With the development of new standards, software, assays, and the improved performance of high-resolution flow cytometers, Moore is excited about the future of EV measurements and the potential this has for liquid biopsies for many conditions, "This field is going to explode."

Learn more about using flow cytometry for extracellular vesicle detection here.

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