Corning® Videodrop for a Fast Characterization of Extracellular Vesicles Following a Standard Protocol

CORNING

Application Note

Quentin Sabbagh[1], Mathilde Richard[1], Gwennan André-Gregoire[2], Julie Gavard[2], Laetitia Guevel[1], and Marie Berger[3]
[1]SOAP (Signaling in Oncogenesis, Angiogenesis and Permeability, CRCINA, Inserm, CNRS, University of Nantes; [2]ICO (Integrated Center for Oncology); [3]Myriade

Abstract

The recent interest growth in Extracellular Vesicles (EVs) is governed by the potential that these cell-derived membranous nanoparticles present in terms of theranostic effect. EV science has now clearly achieved widespread development, as demonstrated by the constantly growing number of EV publications, confirming significant roles of EVs in various physiological pathways like aging, cancer, infectious diseases, and others.

Therefore, there rises an urgent need for both analytical/characterization techniques before proceeding to clinical translation. Up to now, EVs quantification and sizing were achieved by Tunable Resistive Pulse Sensing (TRPS), a complex method relatively time-consuming.

Myriade, a French company, developed the Videodrop, a new approach for rapid and easy characterization of nanoparticles in a single drop, based on Interferometric Light Microscopy (ILM).

We compared those two methods for EVs characterization on EVs separated from serum and biological liquids: ILM and TRPS. The correlation between the two methods appears to be robust, with high R^2 values. These results suggest the Videodrop can be a relevant tool for quick characterization of EVs for the study of EVs' role in physiology and pathology. It is an easy-to-use and fast alternative to the standard more complex and time-consuming methods.

Introduction

EVs are cell-derived nanoparticles, from 30 to 1,000 nm, released in the extracellular medium. They represent a physiological intercellular communication mode, both locally and regionally, throughout the organism[1] (Figure 1). As such, EVs have the ability to transport bioactive materials such as proteins, nucleic acids and lipids, from donor to recipient cells in their vicinity and at distance. Beside their fundamental role in cell-to-cell communication in both health and diseases, EVs display the advantage to overcome difficult bio-barriers and immune defenses making them interesting targets for cell-free therapeutic intervention in cancer, and metabolic, autoimmune, and inflammatory diseases, as well as regenerative medicine and pathogen vaccination. As EVs are abundant and stable in body fluids (plasma, urine, breast milk...), they emerged as promising biomarkers for the assessment of health status, but also responses to treatment outcomes in pathological conditions. As EVs are nanosized objects, research on EV requires advanced technologies and specialized expertise to assess both their specificity and sensitivity as biomarkers, as well as their efficacy and safety as therapeutic tools.

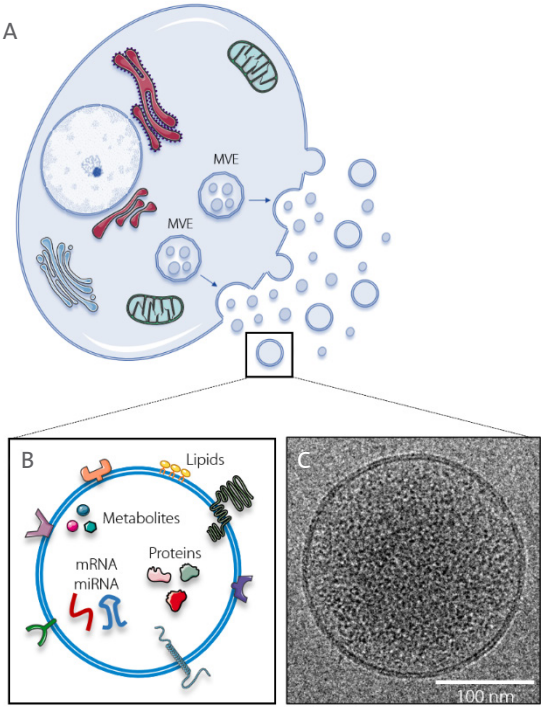

Figure 1. Biogenesis, composition and morphology of EVs. (A) Schematic representation: EVs are virtually released by every cell, either by cell membrane budding or Multi Vesicular Endosome (MVE) membrane fusion. These biologic nanoparticles are highly heterogeneous in size and categorized in two subtypes: small EVs (size < 200 nm) and large EVs (size > 200 nm). (B) Composed of a large, yet specific, range of proteins, nucleic acids and lipids, EVs act as information carriers conveyed from donor to targeted cells, thereby modulating their biology. (C) Plasmatic EVs visualized by cryo-electron microscopy.

In this context, the SOAP (Signaling in Oncogenesis, Angiogenesis and Permeability) laboratory, an academic research team led by Dr. Julie Gavard (CRCINA, Inserm, CNRS, University of Nantes, France), is exploring the role of EVs as hijacked communication tools in primary malignant brain tumors[2-4]. For this purpose, EVs are separated from either plasma samples and urine samples and, up until now, quantified by Tunable Resistive Pulse Sensing (TRPS) using qNano technology (Izon Science). However, a new method for quick and reliable EV quantification, compatible with the analysis of a large quantity of patient samples and with limited volume of liquid biopsies, is required.

Myriade, a French company specializing in Interferometric Light Microscopy (ILM), has developed a new detection method for nanoparticles in solution, the Videodrop, based on ILM. The Videodrop measures in real-time the concentration and size of nanoparticles from a single droplet of sample. The relevance of this method lies in its simplicity: the measurement is label-free, filtration-free, non-destructive, fast, and only requires 5 to 10 µL sample volume. This technology can therefore be particularly adapted for EV characterization.

Here, we compare the quantification of EVs (recently called vesiclemia[5]), by the Videodrop and qNano (Izon Science, NZ), a well-established method for the characterization of EVs separated from serum and biological fluids. After having shown the suitability of the Videodrop for quick EVs quantification, we applied this new technology for a simple stability study to determine the best storage conditions for EVs sample.

Materials and Methods

EV Isolation

EVs were separated from plasma or urine by Size Exclusion Chromatography (SEC) using 70 nm (Original) qEV columns associated with automatic fraction collector (Izon Science) (Figure 2). Both the Videodrop and qNano analyses were performed on fresh EV preparations.

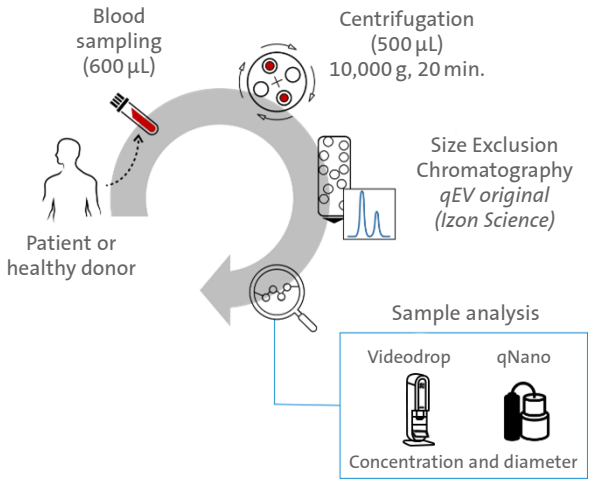

Figure 2. Purification process and quantification of EVs from patient or healthy donor blood.

Interferometric Light Microscopy (ILM)

The Videodrop is a custom microscope that uses interference phenomenon to detect the light scattered by individual nanoparticles in solution. Videos recorded by the Videodrop are processed to reveal the diffraction patterns created by the nanoparticles moving in the light path. Using this interferometric signal, nanoparticles are automatically detected and tracked to compute concentration and hydrodynamic diameter. Counting particles allows to measure the concentration, while tracking their Brownian motion allows to measure their hydrodynamical diameter. The microscope magnification and camera speed allow to perform analysis of small sample volumes (down to 5 µL) in less than one minute.

The instrument is essentially based on a microscope, therefore objects in the micrometer range will be seen, in a similar manner than with a conventional microscope, thus also allowing the evaluation of sample composition (i.e. large or small EVs). This innovative way of detecting nanoparticles through interference allows the analysis of size polydisperse samples without glare nor clogging. Figure 3 shows the diffraction of limited spots of EVs from 50 to 400 nm (median 200 nm).

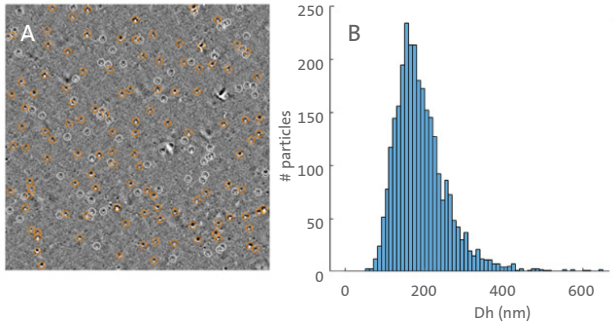

Figure 3. EVs images obtained with ILM (A) Nanometric image where diffraction patterns of EVs can be detected and tracked to compute concentration and hydrodynamic diameter. (B) EVs size distribution histogram from tracked particles data.

In order to optimize inter-operator variability, a standard protocol was developed (Table 1).

Tunable Resistive Pulse Sensing (TRPS)

qNano is an equipment relying on Tunable Resistive Pulse Sensing (TRPS). It is based on the Coulter principle, which states that particles pulled through a pore, while an electric current is applied, produce a change in impedance that is proportional to the volume of the particle travelling through the pore. Samples were analyzed using NP100 nanopores (Izon Science), after prior dilution (1/4) in 0.22 µm-filtered PBS. Measurements were performed on a 35 µL volume, from diluted samples.

Stability Study

In order to determine storage conditions for a standard protocol, a sample stability study was carried out at different temperatures for three weeks. EVs were prepared from one biological sample and stored at +4°C, -20°C and -80°C. Concentration measurements were performed after two and three weeks. The Videodrop was used to evaluate the stability of plasmatic EVs, by following the quantity of EVs before and after storage.

Results and Discussion

EVs are studied for their abilities to transfer bioactive components and for their physiological/pathological functions in cell-to-cell communication[6].

Besides their instrumental role in transcellular message delivery, EVs may quantitatively and qualitatively vary with gender, ageing and diseases[5,7]. Therefore, EVs may represent a valuable and informative resource on health status and disease progression.

The concentration of circulating EVs can be obtained from minimally invasive liquid biopsies and is currently studied in a wide variety of diseases. After establishing rigorous, reproducible methodology to isolate EVs from clinical samples, researchers now need an accurate and rapid characterization method of large quantities of samples.

Videodrop Standard Protocol Development

Objective	Obtain the best performances of the device and optimize the inter-operator variability.
Protocol	1. Samples are systematically diluted in 0.22 µm-filtered PBS (successive dilutions: dilution x2; dilution x4; dilution x8; etc.) 2. The concentration of each dilution is measured with the same image processing settings: threshold 4, saturation between 90 and 95%. Measurements are performed on 7 µL droplets. 3. The suitable and linear range of concentration is identified when the experimental dilution factor is accurate with a 20% error tolerance. 4. The raw concentration is the mean of the dilution-corrected linear values.

3 Linear range identified

1	Raw sample (non-diluted)	Dilution x2	Dilution x4	Dilution x8	Dilution x16
2 Measured concentration (part./mL)	6.49×10^9	6.09×10^9	4.97×10^9	2.50×10^9	Not determined
Experimental dilution factor		1.07	1.23	1.99	
4 Dilution-corrected concentration (prat./mL)			1.99×10^{10}	2.00×10^{10}	

Mean of the dilution-corrected linear value: 2.00×10^{10} part./mL

Table 1. Illustration of the standard protocol developed: example, with three systematic dilutions and the raw concentration calculation corresponding to the mean of two linear values.

Concentration of Nanoparticles

EVs were separated from 6 plasma samples and 4 urine samples using SEC and analyzed upon their separation, before storage.

Vesiclemia was estimated by ILM using the Videodrop and following a standard protocol and TRPS using qNano. The Videodrop and qNano display a linear correlation regarding EV concentration (correlation coefficient $R^2 = 0.95$) (Figure 4).

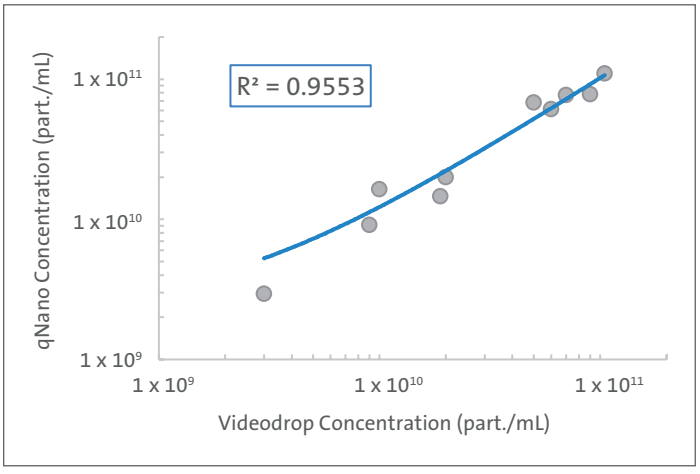

Figure 4. Comparison of the Videodrop and qNano quantification methods on EVs extracted from biological liquids (plasma n=6 and urine n=4

Size Distribution of Nanoparticles

The Videodrop provides information of concentration as well as size of nanoparticles in solution. The size of each detected nanoparticle is obtained from the calculation of its trajectories. The size result can thus be used for EVs characterization.

Figure 5A shows the profile of EVs from a representative sample of plasma or urine, measured by the Videodrop. Figure 5B shows the mean values of several samples, indicating a difference in the size and distribution of EVs isolated from plasma and urine samples. EVs isolated from urine seems to show higher hydrodynamic diameter than EVs isolated from plasma.

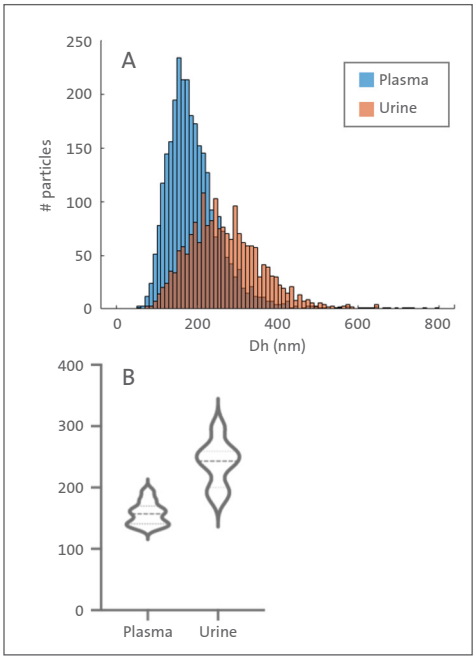

Figure 5. Comparison of size between EVs from plasma and urine samples by the Videodrop.

Storage Conditions

Stability of EVs and storage conditions (temperature and duration of storage) are always a challenge for biologists. EVs from biological samples need to be stored for a long period of time and researchers should be able to confirm the comparability of samples after the storage.

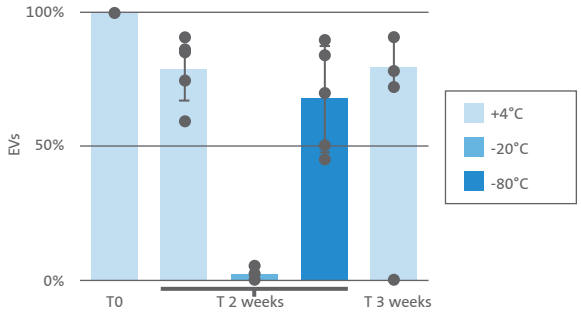

Figure 6. Storage stability at 4°C after 2 and 3 weeks, at -20°C and -80°C after 2 weeks

In this stability study, EV fraction shows a loss of less than 20% after storage at +4°C for 3 weeks and at -80°C for 2 weeks. However, after storage at -20°C EVs are no longer measurable by the Videodrop (Figure 6). This may be due to a loss of integrity of EVs. Based on this data, EVs should be preferentially stored and quantified by the Videodrop after freezing at -80°C.

Conclusion

In this comparative study, the Videodrop and qNano technologies were used in parallel to quantify plasmatic and urinary EVs collected from 10 individuals. The development of a rigorous and reproducible measurement protocol for the Videodrop allows to obtain reliable size and concentration values. The results obtained with the Videodrop regarding EV concentration correlate well with the ones from TRPS (qNano), with a correlation coefficient of $R^2 = 0.95$.

The Videodrop can further distinguish a size difference in between two types of EVs population (plasma and urine). The Videodrop can be used as a tool for quick characterization of EVs sample size.

In both fundamental and translational research, but also clinical trials, a systemic standardization to better assess the concentration of EVs is highly required. In this context, the Videodrop is particularly well suited to fulfill the pre-requisite for EVs characterization, as non-invasive biomarkers. Consequently, this fast, real-time titration method, requiring minimal sample volumes, turns out to be suitable for EV quantification.

References

1. Théry C, et al. Minimal information for studies of extracellular ISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. Journal of Extracellular Vesicles. 2018;7(1):1535750.
2. Treps L, et al. Extracellular vesicle-transported Semaphorin3A promotes vascular permeability in glioblastoma. Oncogene. May 2016;35(20):2615-23.
3. André-Grégoire G, Bidère N, Gavard J. Temozolomide affects Extracellular Vesicles Released by Glioblastoma Cells. Biochimie. 1 Dec 2018;155:11-5.
4. Treps L, et al. Glioblastoma stem-like cells secrete the pro-angiogenic VEGF-A factor in extracellular vesicles. Journal of Extracellular Vesicles. 1 Dec 2017;6(1):1359479.
5. Sabbagh Q, et al. Vesiclemia: counting on extracellular vesicles for glioblastoma patients. Oncogene. 2020;39(38):6043-52.
6. Van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. Nature Reviews Molecular Cell Biology. April 2018;19(4):213-28.
7. André-Grégoire G, Gavard J. Spitting out the demons: Extracellular vesicles in glioblastoma. Cell Adhesion & Migration. 4 March 2017;11(2):164-72.