Resistive Pulse Sensing with Micro-fabricated Nanopores (NP-RPS) for Sub-micron Biomolecule and Bionanoparticle Analysis

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Abstract

Biomolecules and bionanoparticles, such as nucleic acids, proteins, microorganisms and extracellular vesicles (EVs), are recognized as important targets for fundamental research, clinical diagnostic and therapeutic applications. To gain detailed information of those bionanoparticles, we demonstrate an electroosmotic (EO) driven transport behavior in silicon and silicon nitride-based nanopore, towards an accurate measure of concentration and sizing of sub-micro particles for a general biological interest.

Introduction

The resistive pulse sensing (RPS) strategy, so called coulter principle, has been extensively exploited in cell sorting and counting back to 50's. Benefitted from modern micro and nano fabrication techniques, several powerful and effective methods and systems have been applied for sensitive, high-throughput and high definition characterizations of sub-micro scale objects, such as nanoparticles[1], virus[2], ions[3], and DNA[4,5]. Toward various categories of sensing targets, the target sizes span a wide range from several nanometers to several hundred nanometers. Biomolecules and bionanoparticles, such as nucleic acids, proteins, microorganisms and extracellular vesicles (EVs), are recognized as important targets for fundamental research, clinical diagnostic and therapeutic applications. Those biological objects, in principle, can be identified and categorized into sub-populations by their intrinsic parameters, such as size, surface charge or even shape. Sorting biomolecules based on their physical properties is of vital importance. Recently, Extracellular vesicles have been implicated in trafficking of molecules between cells and as such have an effect on physiologic function and serve as biomarkers for disease[6].

RPS is a single-particle technique that has been shown to measure the particle size distribution (PSD) and concentration of synthetic and polymeric nanoparticles. This technique has been widely used to sort different types of blood cells, yet limited to the micrometer as the lowest detection scale. As an alternative approach, flow cytometry (FCM) has been used to detect extracellular vesicles and populations of sub-micron nanoparticles. To get sizing information, but also to directly visualize particle morphology, electron microscopy approaches such as scanning electron microscopy (SEM) and transmission

electron microscopy (TEM) are the techniques of choice. In terms of automation and time efficiency, the utilization of electron microscopy is still limited within the research field. To study the ensembles of nanoparticles, dynamic light scattering (DLS) techniques are the golden standard to date. However, DLS is lack of the capability of measuring the concentration of particles. Nanoparticle tracking analysis (NTA) is another light scattering technique, based on the analysis of Brownian motion of single particles. It can measure particle size distribution and concentration simultaneously.

Here, we demonstrate an electroosmotic (EO) driven transport behavior in silicon and silicon nitride-based nanopore, towards an accurate measure of concentration and size of sub-micro particles. In details, as illustrated in Figure 1, an "anomalous" translocation behavior through pores in silicon nitride (SiN_x) membranes has been reported and was also manifested by the phenomena: positively (negatively) charged objects transported through the pore toward the positively (negatively) charged electrode. Clearly, the direction of these translocations cannot be understood by electrophoresis, so we assumed that EO effects might be involved. EO flow occurs in micro- and nanochannels with charged sidewalls: an electric field applied along the channel sets the electrical double layer (DL), which screens the surface charge, in motion. The DL drags the fluid along and as a result a net flow is created which can be represented with the electroosmotic velocity v_{EO} given by eq 1, where $\eta=10^{-3}$ Pa s, $\varepsilon = \varepsilon_0\varepsilon_r$, ε_r =80;E is the electric field, and ζ_{pore} is the pore potential.

$$v_{EO} = -\frac{\varepsilon}{\eta}\zeta_{pore}E \qquad \text{eq 1}$$

Practically, a steady flow can be obtained in a pore for a precise concentration measurement. The counted frequency is proportional to the flow rate and the concentration of measured particles. Moreover, the flow rate can be quantified using a diluted solution with a known concentration of particles. The height (Δi) of the current pulses provide information about the volume of single particles, which can be used to obtain the diameter (dsnp) of spherical particles. Here, lc' is the channel length, after correction for end effects (lc' = lc + 0.8dc) and S(dc, ds) is a correction factor that depends on the relative values of dc and ds.

$$\Delta i \,/\, I = S(d_c, d_s) * d_{snp}^3 \,/\, l_{c'} * d_c^2 \qquad \text{eq 2}$$

Experiment sections

Nanopore membranes fabrication. The SiN_x membranes are prepared using the previously reported procedure. Briefly, 20-nm-thick supporting SiN_x membranes are manufactured using anisotropic KOH etching to obtain 100 µm × 100 µm to 500 µm × 500 µm membranes, with size depending on the size of the backside opening. Reactive ion etching (RIE) is used to make a 50 nm-10µm opening on that membrane.

Here, we use a two-part flow cell based on computer numerical control (CNC)-machined PMMA. The flow cell comprises two PMMA blocks that can be screwed together. The nanopore chip is sandwiched between two rubber O-rings placed in the appropriate grooves in the PMMA blocks. Conventional reference Ag/AgCl electrodes are used. In addition to having a stable electrode potential, they are also nonpolarizable, which means there is no need to overcome an overpotential in order for the current to flow. In other words, in this way, the potential difference across the membrane is equal to the potential difference that

is applied. Current is measured in voltage-clamp mode with an integrated data acquisition system.

Comparison with other Tools for Determining Nanoparticle Size. The diameter of individual nanoparticles in an aqueous solution can be calculated from the ratio of signal height to background current (eq 2) determined using NP-RPS. We compared the diameter of nanoparticles obtained using NP-RPS (d,RPS) to those obtained using TEM (d,TEM) and DLS (d,DLS). In both the NP-RPS and TEM methods, each particle is sized individually. All the tested nanoparticles have been checked routinely by TEM, as shown in Figure2a. It is time-consuming and has low statistics in terms of the counted particle number. For example, it takes two hours to get few images containing few hundreds of particles. In contrast, as shown in Figure 2b, thousands counts of single particles can be achieved in minutes. A histogram of particle sizes can be updated in real-time while online measuring. For each of these methods, Table 1 reports the averages and standard deviations of the particle diameters from an analysis of more than 100 individual particle measurements. Here we demonstrate an agreement with the other two techniques in sizing the nanoparticles.
Moreover, as demonstrated in Figure2d, a true measure of the polydispersity of the nanoparticles has been shown possible only by NP-RPS. A clear four peaks distribution demonstrates the capability of the NP-RPS method in revealing the heterogeneity of the polydispersed nanoparticles. The measurement only takes 10 minutes to get a statistical histogram, which is superior to the time consuming SEM measurement.

Precision in measuring the concentration of nanoparticles

Here we compare the performances of measuring the concentrations of nanoparticles among three different methodologies. It is worth noting that, to date, there has been no golden standard to quantify the exact concentration of sub-micrometer nanoparticles in their pristine condition (in aqueous condition). The state-of-art Coulter principle has been widely exploited as a golden standard in measuring the exact concentration of the particles or blood cells with dimensions above a few micrometers. Recently, there have been some new techniques to tackle the sorting and counting problems under sub-micrometer scale.
Nanoparticle tracking analysis (NTA) is an optical method to track single particles and to count the exact number of the objects by a direct visualization of tracked single particles within a confined volume (typically on the order of nano-liter). Nanoflow cytometry measurement (NFCM) is a flow-based technique that detects nano-sized particles by scatter and/or fluorescence. Compared with traditional flow cytometry, a smaller flow channel reduces background signal, and lower system pressure increases dwell time of particles for enhanced signal integration. From the evaluation results, NTA gives a significant discrepancy for two types of particles with similar sizes but with different surface modifications. It can be assumed that the physical properties of particles can influence the Brownian motions in the liquid, thus the calculated size should take the surface property of measured particles into account.

Conclusions

Here we compare the performances of sizing and measuring the concentrations of nanoparticles among different methodologies. NP-RPS shows advantages in automation, label-free, and time efficiency, especially it gives way more information of the heterogeneity of the sample than other methods. To advance the fast development of nanomedicine, and nanovaccine, NP-RPS can be a powerful tool for detecting and characterizing biological materials such as viruses, biomarkers, and DNA under physiological conditions.

References

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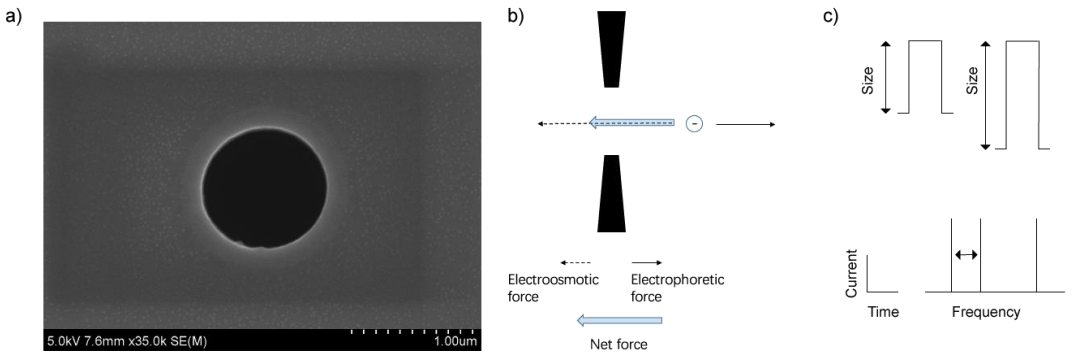

Figure 1. a) SEM image of a micro-fabricated pore on membrane. The scale is 1 μm. b) The scheme of the transport influenced by multi-forces. c) A schematic illustration of the signals.

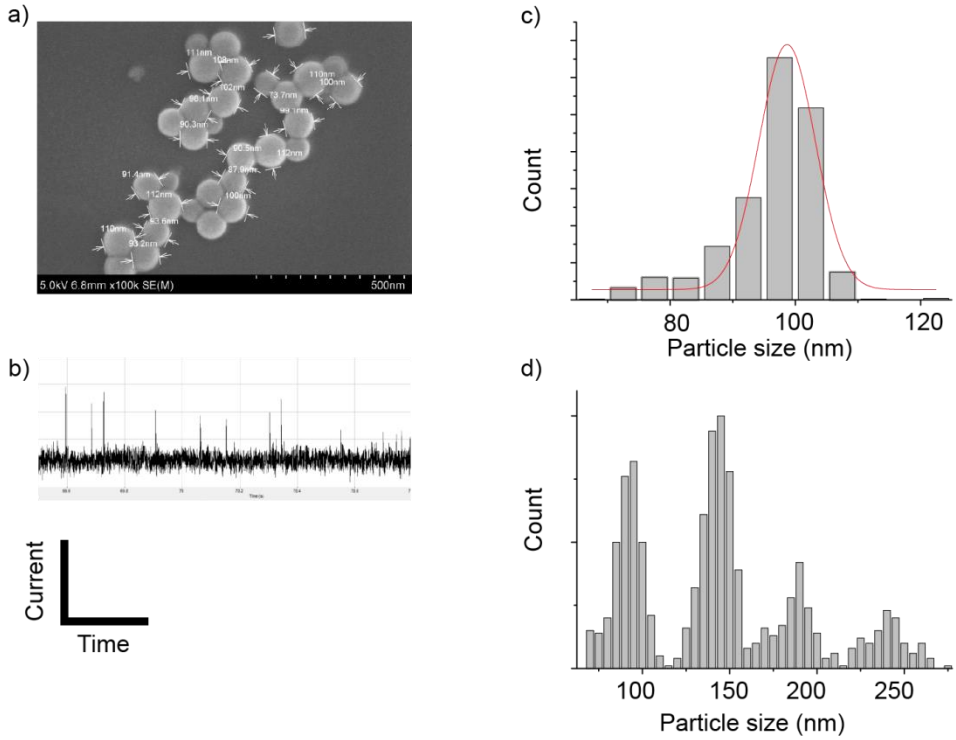

Figure 2. a) Standard SEM image of nanoparticles with a nominal size around 100 nm. b) Recorded current versus time I(t) trace. c) A histogram of particle size distribution generated from current data and calculated by eq 2. d) A histogram of particle size distribution of mixed four types of nanoparticles.

Sample#	DLS Diameter (nm)	SEM Diameter(nm)	NP-RPS Diameter (nm)
1#	109.8	98.8	102
2#	247	217.6	219
3#	128	119.8	126
4#	185.4	194	198
5#	242	228.7	224

Table 1. Comparison of performance in sizing nanoparticles with single distribution among three platforms.

Sample#	SEM Diameter (nm)	NP-RPS Diameter (nm) and concentration (particles/mL)	NTA Diameter (nm) and concentration (particles/mL)
1#	164.6	159，8.92E+11	102，3.62E+11
2#	165.5	160，1.05E+12	219，3.50E+11
3#	180/405.5	166/390，2.21E+11	NA

Table 2. Comparison of NP-RPS and NTA in sizing nanoparticles and .measuring concentrations. Sample #3 has a dual distribution.