NANOPORES WITH ASYMMETRIC SPACING FOR RESISTIVE-PULSE SENSING OF VIRUS PARTICLES

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ABSTRACT

We report resistive-pulse sensing of hepatitis B virus (HBV) capsids in nanochannels that have three nanopores in series and with asymmetric spacing. Two or more pores in series are able to interrogate single particles multiple times for improved measurement of particle sizes and their electrophoretic mobilities. With asymmetric spacing of the pores, each particle produces a three-pulse signature that is easily tracked at high particle concentrations. Moreover, the in-plane format permits systematic evaluation of nanopore dimensions (e.g., cross-section and length) and pore-to-pore spacing for optimized particle characterization.

KEYWORDS: resistive-pulse sensing, nanofluidics, hepatitis B virus, nanopores

INTRODUCTION

Resistive-pulse sensing techniques measure the change in conductivity as an analyte transits a pore of comparable dimensions [1]. Multiple pores can be arranged in series to probe a single particle multiple times. In prior work, Rant et al. formed two nanopores in separate membranes and stacked the membranes out-of-plane [2], whereas we fabricated in-plane devices with two nanopores in series [3]. Fabrication of nanofluidic devices in-plane allows us to arrange the nanochannels in any arbitrary format and tune the number and dimensions of the pores to study individual virus capsids and their assembly.

EXPERIMENTAL

We fabricate two V-shaped microchannels in a glass substrate by conventional techniques, then nanochannels with integrated nanopores are milled with a focused ion beam (FIB) instrument into the substrate to span the microchannels (see Figure 1). An electron flood gun assists the ion beam during the mill, circumventing the need to coat the substrates with a metal film. Device geometry is characterized by scanning electron microscopy and atomic force microscopy, which benefits data interpretation as particle translocation events represent the convolution of particle and nanopore geometry. Nanopores that are 50-nm wide and 50-nm deep are fabricated and customized to match the sizes of the 31-nm diameter T = 3 and 36-nm diameter T = 4 HBV capsids. A potential of 100–1000 mV is applied across the nanochannel to drive the capsids through the pores, and the resulting current signal is amplified and digitized for analysis.

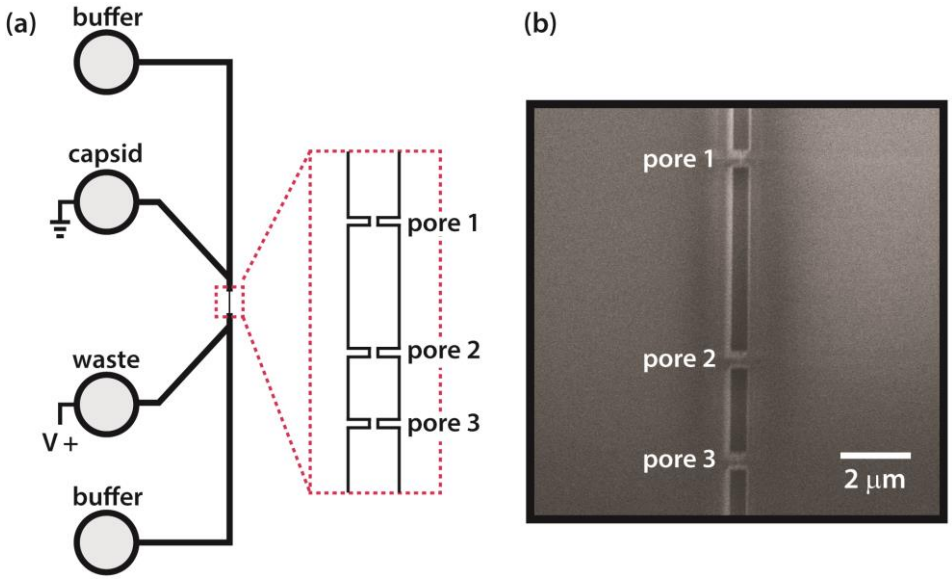

Figure 1: (a) Schematic of two V-shaped microchannels bridged by a nanochannel that has three nanopores in series and with asymmetric spacing. Inset is the expanded view of the nanochannel with pores 1 and 2 spaced 5.5 μm apart and pores 2 and 3 spaced 2.5 μm apart.(b) A scanning electron microscopy (SEM) image of the three-pore nanochannel used to generate the data in Figures 2 through 4.

RESULTS AND DISCUSSION

For the resistive-pulse measurements, HBV capsids are electrokinetically driven through the nanochannel, and as a capsid transits a pore, a decrease in current (pulse) is observed. Three current pulses correspond to the passage of a single capsid through a three-pore nanochannel (Figure 2). The three-pulse sequence generated by each capsid aids in pulse identification at high particle concentrations and at low signal-to-noise ratio.

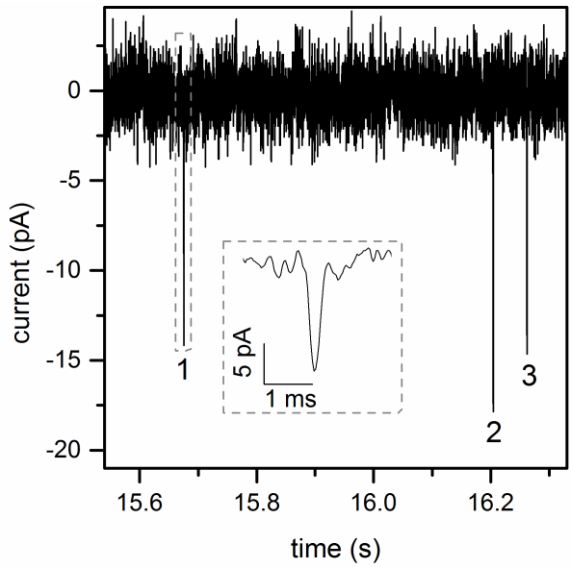

Figure 2: Variation of current with time through three nanopores with asymmetric spacing. Each HBV capsid generates three current pulses while transiting the three nanopores. Inset is the expanded view of pulse 1.

Pulse amplitude increases with increasing potential, whereas pulse width and the time between pulses both decrease. Pulse amplitude is proportional to the capsid size, and pulse width is used as a measure of the capsid-pore interaction. The time between pulses is used to calculate capsid velocity, and subsequently, electrophoretic mobility. An advantage of the resistive-pulse sensing technique is the ability to discriminate between subtle changes in analyte size. A 5-nm difference in HBV capsid diameter is easily resolved in pure and mixed populations, as shown in the histograms of pulse amplitude in Figure 3. The larger, 36-nm diameter capsids displace 9.6 pA of current on average, whereas the smaller, 31-nm diameter capsids displace 7.9 pA on average.

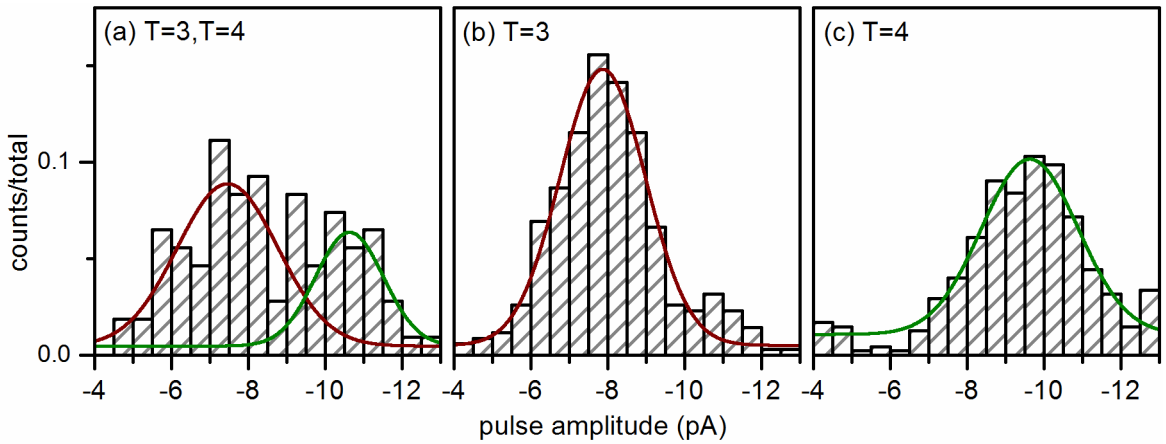

Figure 3: Histograms of pulse amplitudes from the three-pore resistive-pulse sensing of (a) a 1:1 mixture of T = 3 and T = 4 capsids, (b) 31-nm diameter T = 3 capsids, and (c) 36-nm diameter T = 4 capsids

The velocities of the capsids are measured from the pore-to-pore transit times between pores 1 and 2 and between pores 2 and 3. Velocity increases with applied potential, as capsids travel faster in higher electric fields (Figure 4). As the capsids are electrophoretically driven through the nanochannels, the relationship between field strength and velocity is linear, and the pore 1-2 velocity and pore 2-3 velocity are comparable in magnitude. This presentation will include in-depth characterization of the effects of pore size and spacing on particle transit and will discuss the forces acting upon the particles at the entrance and within the pores at different applied potentials.

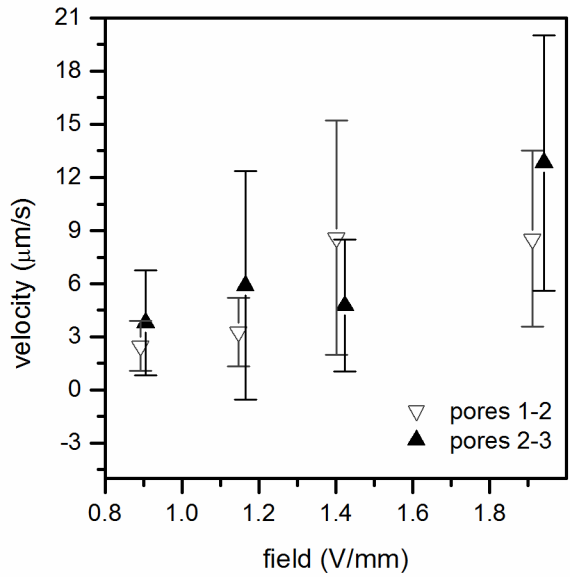

Figure 4: Variation of velocity with potential for T = 4 HBV capsids traveling from pore 1 to pore 2 (5.5 μm apart) and from pore 2 to pore 3 (2.5 μm apart).

CONCLUSION

Three asymmetrically-spaced pores in series are used to simultaneously measure the sizes and velocities of hepatitis B virus capsids in solution. Fabrication of the nanopore devices in-plane permits a great degree of flexibility in device layout, such that a variety of unique structures may be tested and optimal device geometry be sought.

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