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The Effects of Geometry and Stability of Solid-state Nanopores on Detecting Single DNA molecules

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Abstract

In this work we use a combination of 3D-TEM tomography, energy filtered TEM, single molecule DNA translocation experiments, and numerical modeling to show a more precise relationship between nanopore shape and ionic conductance and show that changes in geometry while in solution can account for most deviations between predicted and measured conductance. We compare the structural stability of Ion Beam Sculpted (IBS), IBS-annealed, and TEM drilled nanopores. We demonstrate that annealing can significantly improve the stability of IBS made pores. Furthermore, the methods developed in this work can be used to predict pore conductance and current drop amplitudes of DNA translocation events for a wide variety of pore geometries. We discuss that chemical dissolution is one mechanism of the geometry change for SiN_x nanopores and show that small modification in fabrication procedure can significantly increase the stability of IBS nanopores.

Keywords

solid-state nanopore; DNA; nanopore geometry; 3D-TEM tomography

1 Introduction

Research over the last decade has shown the single molecule sensitivity of solid-state nanopores to the study of DNA [1], ssDNA [2], RNA [3], and proteins [4]. The sub-

molecular sensitivity of solid-state as well as their biological counterparts has inspired investigation into their use in single molecule high throughput DNA sequencing with recent success in detecting sequence dependent information within DNA molecules [5, 6, 7]. A typical nanopore experiment places the pore as the sole fluidic and electronic connection between two ionic solution filled reservoirs across which an applied voltage bias drives an ionic current through the nanopore. The current through the pore depends upon the size and shape of the nanopore and is modulated by the presence of single macromolecules that are electrophoretically driven through the pore. For the pores investigated in this work these current modulations typically come as brief reductions in current, the duration and amplitude of which are a function of both the molecule and pore.

Genetically engineered variants of natural transmembrane protein pores have the highest sensitivity to date [5, 6], but the tunable size and shape of solid-state nanopores and their easily scalable integration with wafer scale electronics fabrication make solid-state nanopores more appealing. Typical solid-state nanopores are fabricated through either direct drilling by a TEM-based electron beam [8, 9], or use a combination of drilling and closing a larger hole as in ion-beam-sculpting (IBS) [1], atomic layer deposition [10, 11], and thermal annealing [12, 13]. Materials used to fabricate nanopores include SiO_2 [8], SiNx [1], Al_2O_3 [10, 11], and suspended graphene [14, 15, 16]. Hybrid biological solid-state nanopores include combining transmembrane pores [17], DNA origami pores [18] or lipid bilayers with solid state substrates [19].

Although advances in fabrication have improved the dimensions of solid-state nanopores for their use in single molecule experiments, less work has been done to ensure pores remain at those dimensions in ionic solutions. Recently, increase in conductance during experiment has been attributed to changes in pore radius [12, 20], however, both the diameter and pore thickness are expected to play a role in pore conductance. Plan-view TEM images similar to those in Figure 1A that give the pore diameter and thin film measurements of the membrane thickness supporting the pore are typically the only information used to estimate pore conductance. To included detailed information from the pore thickness profile, we measure the 3D structure of pores both before and after wetting using 3D TEM tomography and energy filtered TEM thickness mapping as shown in Figure 1B and C. We show here that pore radius increases while keeping the general pore thickness profile nearly unchanged. Furthermore, based on the assumption that the slow changes in pore conductance are due to dissolution of the pore, we develop an annealing method to increase the stability of IBS fabricated nanopores.

The reconstruction in Figure 1C, confirms earlier work [30, 29] that show IBS pores have a lateral mass flow at the top surface that produced a single truncated cone at the resolution measured. We note that material in IBS pores flows at a depth greater than the mean penetration depth of the ion [30]. The conical thickness profile creating the transition between nanopore and the larger vestibule is similar to that hypothesized by Cai [30] but is unlike the stressed overhang predicted by George [31]. These results will be important for future modelling of lateral mass flow caused by IBS.

2 Methods

2.1 Nanopore fabrication

To determine the applicability of our method over a wide range of pore dimensions, nanopores were fabricated using either IBS or TEM drilling with diameters between 4nm to 45nm with a median diameter of 12nm. Details of the IBS method [21] and TEM method [9] are documented elsewhere. Briefly, in the IBS method we first use a 50 Ga⁺ focused ion beam (FIB) to mill a single ~100nm diameter hole in a ~250nm thick freestanding low tensile stress, silicon rich LPCVD deposited SiN_x membrane suspended on a 3×3 mm silicon chip deposited with dichlorosilane and NH₃ at 800 °C.

This chip is mounted in a custom made vacuum system [22] where a 3keV Ne⁺ ion beam was directed normal to the SiN_x surface with a typical flux of 1 ion/nm²sec. Ions that passed through the hole were focused by an electrostatic lens system on a ‘channeltron’ style single ion detector connected to a LabView controlled single ion counting system. The ion beam impinging upon the pore elicits lateral mass flow that shrinks the top of the hole. As the hole shrinks, the ion beam current through the hole decreases. By measuring the beginning hole area with TEM and assuming that the pore area is proportional to the ion beam current, the beam was deflected using a Labview controlled feedback system when the desired area was reached. To determine if a decrease in dangling bonds left over from the IBS process can improve the stability of IBS pores, we annealed half of the IBS samples at 800°C for 1 hour in dry N₂ in a tube furnace.

In TEM drilling, we begin with the same freestanding SiN_x membrane and FIB milled a ~200nm deep 100nm diameter pit in the same 250nm thick SiN_x freestanding membrane and drill a nanopore with a 300KeV electron beam of an FEI-Titan TEM in imaging mode focused to spot with width ~3nm. This beam size was used to reduce electron beam induced damage that is known to decrease pore stability [12]. The beam was manually directed to the edge of the pore to widen the pore to the desired size.

2.2 TEM geometry characterization

Analysis of all nanopores were performed using either an FEI-Titan TEM at 300keV or an FEI-Tecnaï TEM at 200keV equipped with Gatan post column electron energy loss filters. Since the contrast of bright field TEM images are difficult to interpret quantitatively, they were used only to determine the radius of the nanopore and vestibule. Energy filtered TEM (EFTEM) at either 300keV or 200keV was used to produce thickness maps of nanopores by taking two images, the first with a 10eV energy slit centered at the zero loss peak (Fig. 1E, shaded area), $I_{zlp}(x, y)$, and another unfiltered image $I_0(x, y)$ and thickness calculated using the log-ratio method,

$$t(x, y) = \lambda \ln \left(\frac{I_0(x, y)}{I_{zlp}(x, y)} \right) \quad (1)$$

These images were aligned using cross correlation to compensate for sample drift using Digital Micrograph (Gatan) [23]. To find the inelastic mean free path for both λ electron beam energies, the thickness of the SiN_x freestanding membrane was measured by thin film

reflectometry. Mean free paths of $\lambda = 185\text{nm}$ at 300keV and $\lambda = 152\text{nm}$ 200keV were found using this method. These values were repeatable from sample to sample within a few nm, well within the 10% error commonly used in measuring very thin structures [23]. Non-zero thickness at the center of the nanopore also seen by other researchers [12] was removed by deconvolution using a point spread function estimated from the EFTEM thickness measurement of FIB milled sharp step edges in membranes of identical thickness and composition as those used to make nanopores. An example cross section from a nanopore is shown in Figure 1B.

To construct our tomograms (Figure 1C), bright field images were zero loss filtered with a filter width of 10eV (shown in Fig. 1(E)) at an acceleration of 200 on an FEI-Tecnaï TEM. Images were taken in 1° increments over $\pm 35^\circ$. Because of the very thick supporting membrane, $\sim 250\text{ nm}$, the thinnest region of the nanopore was obfuscated at tilt angles greater than $\pm 35^\circ$, degrading the resolution of our reconstruction. The tomographic reconstruction of a point in space can roughly be approximated as a 3D ellipsoid [28] with the longest axis, and hence lowest resolution, along the electron beam path. The resulting blurring broadened each point $\sim 10\text{nm}$ along the z-axis but approximately preserves the center of mass of that point in the x-y plane. Image alignment was done using low pass filtering and cross correlation and tomograms were reconstructed using weighted back projection in Inspect3D Xpress (FEI Company). Segmentation was done with Amira 5 (Visualization Sciences Group) using semi-automated thresholding with manual guidance.

The parameters used to analytically model the pore shape were extracted from the radially averaged thickness profile computed using the Radial Profile plugin in ImageJ [24].

2.3 Conductance and conductance drop measurement

Nanopores were mounted between two $\sim 80\text{ }\mu\text{L}$ chambers containing a solution of 1MKCl, 10mM Tris, and 1mM EDTA at pH 7.5. The chambers were constructed of poly-dimethylsiloxane (PDMS) as detailed elsewhere [21, 25]. The entire system was mounted on a vibration isolated Faraday cage (TMC). A pair of Ag/AgCl electrodes was immersed in the two chambers. An Axopatch 200B and Digidata 1322A (Molecular Devices) integrated system was used to apply a DC voltage and to measure current through the nanopores. The dsDNA added for all translocation measurements was 7kbp (Thermo Scientific, NoLimits). Current traces were recorded by pClamp 9 (Molecular Devices), and files were post-processed using custom software written in MATLAB (Mathworks). Pores were wet by first immersing for several hours to overnight in ethanol, followed by mounting in the PDMS cell and flushing with DI water and then 1MKCl. Typically, current was not present through pores immediately and positive and negative pressure had to be applied manually by syringe with current typically appearing in less than 30 min. Once conducting, IV curves were taken several times during an experiment to ensure linearity. Open pore conductance was determined from baseline current without DNA events divided by the applied voltage ($G_0 = I_0/V = 1/R_0$), usually $V = 120\text{mV}$. To ensure that changes in current were not due to slow electrochemical offsets building up in addition to the applied bias, several times an hour the applied bias was set to zero and current was measured. Any remaining current was deemed to be due to slow drift in the applied voltage due to electrochemical potentials forming

between the electrodes and a small 'offset bias' on the order of a few millivolts was applied until the current returned to zero and future applied biases were summed with this 'offset bias.'

To find conductance drops, tens of thousands of events were recorded at multiple voltages between 60mV and 360mV. Mean current drops of unfolded translocations were plotted vs voltage and fitted using least squares regression in IGOR with the slope of fit used to find the conductance drop. All current drop vs voltage curves were clearly linear. Conductance drop errors were assumed from the error to the least squares fit with the assumption that error was normally distributed.

3 Results and Discussion

3.1 Overview of nanopore geometry

As shown in Figure 1, the nanopores studied in this work contain a narrow cylindrical pore opens into a conical region with roughly a 45° angle to a wider, longer cylinder we call the 'vestibule.' The diameter of the small constriction of the nanopore was controllable by varying the ion beam dose and flux as referenced in the methods section, but the thickness was dependent on the pore diameter, and the IBS parameters used. The vestibule diameter and SiNx membrane thickness were kept close to 100 nm and 250 nm for all pores in the study. We use this idealized geometry to model pore conductance later.

3.2 Conductance increased and current drop decreased with IBS made pores not annealed

For the IBS pores that were not annealed, after the pores were filled with salt solution (wet) and the conductance had reached an expected value estimated by their pore radius, we observed the pore conductance kept slowly increasing in most pores before and after the addition of DNA. Pores in salt solution under applied voltage for several hours before the addition of DNA consistently had higher conductances and lower current drop amplitudes than in pores where the DNA was added immediately. Because DNA molecules can permanently clog a nanopore that makes the open pore conductance decrease, it was difficult to quantitatively measure the decrease in current drop of DNA translocation events and the increase in open pore conductance consistently in the same pore. To characterize this phenomena in the same pore, a 12 nm diameter pore (cross section in Figure 2A) was treated to a combination of KOH etching and dewetting to intentionally increase pore conductance and perform DNA translocations in several stages as shown in Figure 2. Figure 2B shows concatenated DNA translocations that were measured before any modification of the pore. To increase pore conductance, 100 mM KOH was added for several minutes and then flushed copiously with Tris buffered 1MKCl, and DNA was reintroduced to the pore. The observed conductance was nearly 5 times higher and the current of DNA translocation events were nearly one fifth their original value, as shown in Figure 2C. This pore was resistant to further KOH etching, so a bubble was rapidly introduced to the fluid chamber to rapidly dewet and wet, rupturing some of the remaining material and increasing the pore conductance by a further 60%. When DNA was again added, the current drop amplitude was decreased by 70% as shown in Figure 2D. The current/conductance histograms in Figure 2E summarize of the current drop amplitude with 240mV applied voltage at each stage:

325±37pA, 131±33pA, and 39±8.5pA at $G_0=40.3\text{nS}$ (9.67nA, initial), 190nS (45.6nA, after etching), and 317nS (76.1nA, dewetting), respectively.

Translocation duration histograms inset within the Figure 2E show that translocation time increased as open pore conductance increased with times of: 198±70μs, 243±96μs, and 279±94μs. This time increase could be caused by the increase in nanopore thickness as the pore diameter had increased (discussed in Fig. 3 and Fig. 4), and an increase in the pore thickness will decrease the electric field strength in the pore so that the DNA translocation speed will be decreased. In addition, as current drop decreased, our ability to distinguish short events diminished and we were unable to identify events shorter than about 100μs for the translocations through the etched and dewetted stages, biasing our estimates toward longer times. All means and standard deviations of the distributions ($\pm$ values) were calculated from gaussian fits to the appropriate histograms.

3.3 Nanopores etch laterally during conductance increase

To determine what changes in nanopore structure during the conductance increase, we used EFTEM to map the thickness of all pores used in this study. This allowed us to compare their precise geometry with the measured conductance and conductance drop. EFTEM thickness mapping takes only a few minutes and is fast enough for routine measurement, but does not provide the location of the material along the pore axis. Each pixel in the map can be viewed as a column of material of known thickness that could be moved arbitrarily along the z-axis. In order to find the location of the center of mass along the z-axis for each pixel, we used TEM tomography [26, 27] to find the low resolution (~10nm) full 3D reconstructions for several samples fabricated by IBS as shown in Figure 1C.

From the information provided by the tomogram, we were able conclude that the 3D geometry as shown in Figure 1C is similar to the geometry inferred by the EFTEM thickness map when plotted as in Figure 1B, allowing us to approximate our 3D geometry directly from our use of EFTEM maps sufficiently for this work. We were unable to resolve the rounded edge of the pore as shown by destructive cross sectional imaging done recently by Kuan [29]. It is likely that these structures will be important in the conductance modeling for nanopores smaller than those used in this work.

Because our TEM fabricated pores were made from pits of dimensions similar to the holes used to fabricate our IBS pore, they had similar structures at this resolution but consistently larger cone angles. We expect that the region immediately adjacent to TEM fabricated pores has the well characterized truncated double-cone geometry as shown in earlier work [9], but because of the low resolution tomographic reconstruction were unable to resolve the structure.

As shown in Figure 3, after wetting and DNA translocation experiment, most mass loss occurred in IBS pores near the pore and cone. Most mass loss occurred at the thinnest region of the pore, changing the radius of the pore while keeping the cone angle nearly the same. The vestibule and membrane far from the pore showed little change after wetting.

3.4 Determine pore radius change from conductance and conductance drop

The IBS nanopore structure is very different from the cylindrical [32], hyperbolic [33], or double cone [9] pores piercing a flat plane that are the dominating features of most TEM fabricated pores. To account for these differences, we constructed a model as shown in Figure 4A. We describe the geometry as a pore-cone-vestibule structure that can be modelled as a stacked (FIB hole) vestibule cylinder (r_v), cone, and a cylinder with the nanopore radius r_p as shown in Fig. 4A. To model the change in geometry with time, we assume that all mass loss occurs radially at the thinnest region adjacent to the pore. Although the mass loss occurs over the entire cone, changes at the cone region maintained the same angle, making changes in the pore radius r_p have the largest effect on conductance.

For the 1M KCl salt concentration used in this study, the Debye length is $\sim 0.3\text{nm}$, far less than the dimensions of the features of our nanopores, making the Ohmic approximation to conductance reasonable [34, 32, 35]. To approximate the non-cylindrical geometry of the entire structure, we approximate the resistance of our system as the sum of five resistors in series. To find the resistance between the nanopore mouth and the bulk $R_{a,p}$ and the resistance between the vestibule and the bulk on the opposing side $R_{a,v}$ we use the same model as Hall [36]. To find the resistance of the nanopore cylinder R_p , cone region R_c , and vestibule cylinder R_v we approximate the conductance of each separate region using

$$G_0 = \sigma \left(\int \frac{dz}{A(z)} \right)^{-1} \quad (2)$$

where σ is the measured bulk solution conductivity and $A(z)$ is the cross sectional area at point along the pore axis. This approximation is only exact for cylinders with field lines parallel to the pore walls but is within 10–20% of the numerically calculated value for a conical resistor with dimensions similar to ours [37]. Our total pore conductance can thus be written as

$$G_0(r_p) = (R_{a,p} + R_p + R_c + R_v + R_{a,v})^{-1} \quad (3)$$

$$= \sigma \left(\frac{1}{4r_p} + \frac{t_p}{\pi r_p^2} + \frac{t_c}{\pi r_p r_v} + \frac{t_v}{\pi r_v^2} + \frac{1}{4r_v} \right)^{-1} \quad (4)$$

where t_p , t_c , and t_v are the respective thicknesses of the pore, cone, and vestibule, and r_p and r_v are the respective radii of the pore and vestibule. We mathematically model our simplification that etching occurs at only the thinnest region by assuming that t_p increases at the same time r_p increases such that the cone angle is kept the same as shown in 4A. This assumption allows us to model the change in pore thickness t_p and cone thickness t_c as

$$\Delta t_p = - \Delta t_c = \frac{\Delta r_p}{\tan(\theta)} \quad (5)$$

and results in r_p as the sole free parameter to compare our measured conductance with the TEM measured geometry. Theoretically predicted open pore conductances (G_0) and conductance drops (G) vs pore radius (r_p) are diagrammed in Figure 4B and 4C.

Single DNA molecules longer than about 1kbp translocating through the pore produce changes in conductance nearly proportional to the cross section of the molecule [38, 39] in a manner that depends upon pore radius and the relative contribution of the pore and access resistance [33]. Using the Kowalczyk modified Hall access resistance [33] for a long molecule entering a pore and performing the integral in equation while taking into account the blocking DNA molecule, the conductance while the pore is blocked becomes

$$G_b(r_p) = \sigma \left[\frac{1}{4r_{pe}} + \frac{t_p}{\pi r_{pe}^2} + \frac{t_c}{2\pi(r_v - r_p)r_d} \ln \left(\frac{(r_v - r_d)(r_p + r_d)}{(r_v + r_d)(r_p - r_d)} \right) + \frac{t_v}{\pi r_{ve}^2} + \frac{1}{4r_{ve}} \right]^{-1} \quad (6)$$

where $r_{pe} = \sqrt{r_p^2 - r_d^2}$ and $r_{ve} = \sqrt{r_v^2 - r_d^2}$ are the effective radius of a cylinder with the same cross sectional area as the cylinder minus the DNA molecule for the nanopore and vestibule respectively. The third term represents the cone region resistance and is calculated using Eq. (2) where the cross sectional area now takes into account the DNA radius, r_d . Finally, the conductance blockage is calculated with $r_d = 1.1\text{nm}$ for the DNA radius

$$\Delta G = G_0 - G_b \quad (7)$$

We note that in the limit of r_p approaching r_v , equations (4) and (7) simplify to equations modeling the nanopore of a single cylinder used in the literature [40, 33]. The DNA radius of $r_d = 1.1\text{nm}$ can be verified from our data in Figure 2. By using Ohm's law, Eq. (3) and (4) can also be written as $G_0 = I_0/V = 1/R_0 = \sigma A_p/H_{eff}$, where H_{eff} is the effective thickness of a nanopore. Thus we have $H_{eff} = \sigma A_p/G_0$. Using the data from Fig. 2B, $H_{eff} \sim 30.8\text{nm}$ for the

12nm pore was calculated. Further using approximation, $\frac{\Delta I_b}{I_0} \cong \frac{A_{DNA}}{A_p}$, we can estimate the cross-section area A_{DNA} and then the radius of the DNA is $r_d = 1.1\text{nm}$, consistent with our previous estimations.

Both equation (4) and (7) were solved numerically to provide independent estimates of r_p at the moment that DNA translocation events were measured. All pores in Figure 4 are included, for a total of 12 translocation and current drop measurements made on 9 pores. Although on average our pores had conductances slightly higher than predicted from TEM images once wet, several pores had conductances lower than expected from our model. For these, we fit the radius as smaller than measured from TEM images. It is possible that this is due to errors in our measurement of pore geometry and our modelling, but we cannot rule out the possibility of a partial wetting of the pore. Exact agreement between the estimated change in radius from Eq. (4) and Eq. (7) would result in a fit line with a slope of 1 in Figure 4D, but our slope is 1.9 ± 0.2 . Since the change in radius predicted by the open pore conductance and Eq. (4) agrees well with post-wet TEM images, we interpret this result as a systematic under-prediction of the conductance drop magnitude by our model when $r_p > 20\text{nm}$. Wanunu [40] found similarly higher than predicted conductance drops and added a constant parameter to conductance models similar to those used here to accounting for DNA-induced increases in conductivity within the pore. Since the conductance drop is most sensitive to the pore geometry at the narrowest constriction, this may be evidence that our method does not sufficiently model this region at high enough resolution, however, we

cannot rule out other effects due to our assumption that the conductivity in the pore is the same as the bulk.

3.5 Conductance stability for IBS, post IBS annealed, and TEM drilled pores

To compare the stability of IBS, post IBS annealed, and TEM made pores, we recorded their open pore current about 2 hours after the current reached the value estimated from their TEM images. Using Eq. (4), we estimated the rate at which the pore radius r_p changed. Two sets of data from each category for a total of 6 samples are shown in Figure 5 and summarized in Table 1, for the two IBS made pores, the pore radius increased significantly. For the two post annealed IBS pores, the pore radius increased slightly over the 2 hours testing period. This results demonstrate that annealing IBS nanopores significantly increased their stability and made them comparable to and even more stable than TEM drilled pores which were often made from stoichiometric Si_3N_4 .

3.6 Chemical mechanism for variability in etch rate and comparison with the bulk etch rate

In the semiconductor manufacturing industry, SiN_x can be typically etched with aggressive etchants such as phosphoric acid, potassium hydroxide, and hydrofluoric acid [41]. Because of the the long-term stability of microelectronics in less aggressive environments such as implants or environmental sensors is of great concern for MEMs [42], the dissolution of SiN_x in deionized water [43, 42, 44, 45, 46] and salt solutions [47, 48] has also been studied. The chemical reaction of stoichiometric Si_3N_4 with water can be summarized as [45, 44]

In reaction (9) the Si-N bond is hydrolysed by water to produce SiO_2 and ammonia. The SiO_2 further hydrolyses to form silicic acid which diffuses away into the solution, exposing the next layer. Extra silicon in our silicon rich nitride hydrolyses and to form SiO_2 , which dissolves as in reaction (9). This reaction has been shown to follow an Arrhenius type relationship with temperature with rates that depend upon the fabrication method, solution pH, and salt concentration. For neutral pH, etch rates range from 0.01 to 0.1 nm/hr with the highly coordinated crystalline SiN_x [46] powders and LPCVD thin films [47, 42] having the slowest etch rates. Salt concentrations such as that found in living organisms [47] and 1M NaCl [48] were shown to increase these rates by up to an order of magnitude.

To compare the etch rates of SiN_x measured using nanopores with those from bulk thin films, we must first consider any affects of the confined geometry and the contribution of surface energy at the nanoscale radii of curvature. Research in nanoporous SiN_x aggregates show that the dissolution reaction is slow enough to be reaction limited rather than diffusion limited [46], and that the products of dissolution diffuse out of the nanopores fast enough for us to assume that the pore solution is the same as the bath solution. Therefore, the dissolved species are far from equilibrium with the still bound atoms and we can neglect the influence of an increases in the surface free energy due to the nanoscale radii of curvature known to increase reaction rates [49]. Furthermore, research on SiO_2 nanoparticles show very little

change in dissolution rate with radius and suggest that radii greater than 0.6 nm have etch rates that can be predicted by bulk equations alone. Recent work with very high electric fields in nanopores [20] suggest a etching effects due to electric fields at almost two orders of magnitude greater than those used in this work. We thus conclude that the rate of change in radius is directly comparable to the bulk etch rate for TEM drilled and post IBS annealed pores.

It has been shown that annealing silicon oxynide films decreases dangling bonds and increases Si-N coordination [50] and has been shown to reduce the etch rate of LPCVD nitrides in DI water [42] and aggressive wet etchants [51], the significant reduction in etch rate in annealed IBS samples is consistent with a reduction in dangling bonds and reduced sensitivity to hydrolytic attack. We thus conclude that the reason for the increase in dissolution rate for our nanopores over other nitrides in similar salt solutions is due to the presence of dangling bonds that are left over from the IBS fabrication process. This is likely to be the reason why we see greater etch rates for the pore than for the supporting SiN_x film. This conclusion is further supported by the fact that annealing IBS pores brings their radius change rate comparable to that of TEM fabricated pores. Because the TEM beam is mostly confined to the region within the pore, it is likely that the material forming the TEM drilled pores is more like the deposited SiN_x .

4 Conclusion

We used the full 3D geometry of nanopores to show that the simultaneous increase in open pore conductance and decrease in single molecule conductance drop are caused predominately by a lateral change in radius, even in complicated non-cylindrical nanopores. Using a model based on this geometry, we measure pore stability and were successful in developing more robust nanopores. This work provides a method that can routinely determine the thickness profile of nanopores to improve predictions of pore current and conductance drop. These methods will be useful in monitoring and improving nanopore reliability and is applicable to solid-state pores fabricated of any material.

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Figure 1.

Geometry of ion-beam-sculpted (IBS) nanopores. (A) Plan view TEM image of IBS nanopore. (B) Thickness map along solid line shown in TEM image (C) 3D tomogram reconstruction of the same pore. (D) Plan view TEM image of the FIB hole before IBS of (A). (E) Electron Energy Loss Spectrum used for Equation (1).

Figure 2.

(A) Starting cross section and (inset) plan-view image of 12 nm pore used for section B-E. Concatenated conductance drops for (B) Just after wetting (C) after KOH etching treatment (D) after dewetting and rewetting. (E) Scatterplot showing conductance drop and translocation time for all events at each stage as well as their correspondence conductance/current drop and translocation time histograms. The applied voltage for all stages was 240 mV.

Figure 3.

(A) Bright field TEM image of a nanopore as fabricated in vacuum and (B) after wetting and DNA translocation experiment. Dotted line shows outline of pore and vestibule before wetting. (C) EFTEM derived thickness profile before and after wetting. To aid in comparison, thickness profiles were radially averaged then mirrored about the center point. Highly variable thickness at the center of the pore is an artifact of the averaging process.

Figure 4.

(A) Idealized cylinder (pore)-cone-cylinder (FIB hole) geometry of nanopores showing the pore geometry change model. (B) Unfiled shapes depict pore radius predicted by Eq. (4) using measured initial pore geometry and open pore conductance once pore is wet and stable. Filled triangles are the post-wet TEM measured pore radii, representing the same sample as the adjacent unfilled triangle. (C) Similar to (B), but pore radius now predicted using conductance drop data and Eq. (7). Filled triangles (▲) correspond to TEM images of samples post translocation and correspond to the adjacent unfilled triangle. Dotted lines in (B) and (C) are from Eq. (4) and (7) respectively, with parameters: pore radius cone angle 45°, vestibule diameter 120nm, membrane thickness 225nm, dsDNA radius 1.1nm. (D) Comparison between pore radius predicted in (B) and (C). Perfect agreement between the two methods would produce a line at 45°. Dashed line is a linear fit to the data.

Figure 5. Increase in pore radius vs time calculated using Eq. 4 for all fabrication methods tested.

Table 1

Pore radius change rate for all fabrication methods tested. Errors are standard deviations.

Fabrication method	Pore radius change rate
IBS	$10 \pm 6.5\text{nm/hr}$
IBS-annealed	$0.22 \pm 0.4\text{nm/hr}$
TEM drilled	$1.0 \pm 1.2\text{nm/hr}$