Differentiating Single Multiple Nanopore Through Conductance Distribution Analysis

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Solid-state nanopore sensors, a type of resistive pulse sensing, achieve optimal signal-to-noise performance with a single nanopore. However, the processes involved in solid-state nanopore fabrication and subsequent measurements frequently lead to the formation of multiple nanopores, posing a challenge for precise detection. To address this issue, here, a novel and expedient technique to verify the presence of a single nanopore on a chip is developed. The methodology includes measuring the nanopore's conductance in solutions of various salt conditions, followed by a comparison of these results against a theoretical conductance model. This comparison is instrumental in distinguishing between single and multiple nanopores. Additionally, the study delves into various factors that influence the conductance curve, such as deviations in pore shape from the standard circle and inconsistencies in pore diameter. This approach significantly enhances the practical application of low-cost nanopore preparation techniques, particularly in scenarios like controlled breakdown nanopore fabrication, where the formation of multiple nanopores is a common concern.

1. Introduction

Solid-state nanopores have been widely studied for detecting biological macromolecules such as deoxyribonucleic acid (DNA),

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proteins, and viruses.^[1] When a biological macromolecule passes through the nanopore, it will cause the conductance change of the nanopore. The diameter and length of the biomacromolecule can be determined by measuring the conductance change and the duration.^[2] Single nanopores have unique advantages. especially in measuring the concentration of biomolecules with optimal signalto-noise performance. Many methods can be used for the preparation of single nanopores, including transmission electron microscopy (TEM), focused ion beam (FIB), and helium ion microscopy (HIM).^[3] These methods tend to be expensive in equipment and high in processing costs, which limit the widespread application of solid-state nanopores.

In recent years, a novel method for preparing nanopores based on controlled dielectric breakdown has received

extensive attention.^[4] This method can easily and cost-effectively fabricate nanopores. However, this method can form multiple nanopores. Although Tabard-Cossa et al. has designed a comprehensive method to minimize the probability of forming multiple nanopores,^[5] it is still meaningful to further clarify whether the formed pores are multiple nanopores. If the prepared nanopore is not a single pore, it will be hard to determine the transport information of the tested biological macromolecules. The porepore interaction may affect the translocation dynamics and capture rates of nanoparticles.^[6] By using the time interval between two consecutive DNAs passing through nanopores to represent the reciprocal of the capture rate, we find that single nanopore and double nanopore chips with similar conductivity have different capture rates for λ -DNA detection under the same potassium chloride (KCl) concentration (Figure 1). Therefore, chips with unknown pore numbers are difficult to quantify the concentration of λ -DNA. (The detailed information can be found in Figures S1, Supporting Information.) Using TEM to determine the number of nanopores is time-consuming and expensive because the location of generated nanopores by dielectric breakdown is unknown. Some studies have used the fluorescence emission method^[7] to judge the number of nanopores, which suits the case where the nanopores are far apart from each other. For nanopores that are close together, it is difficult to distinguish whether the chip contains a single pore due to the limitation of the optical detection limit. Bandara et al. showed that time-dependent experimental conductance measurements could be used to determine pore www.advancedsciencenews.com

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Figure 1. The effect of the number of nanopores on the capture rate of DNA translocating through nanopores. a) The relationship between the capture rate of λ -DNA (Takara, No.3010) translocation and λ -DNA concentration measured by chips with single and double pores (The electrolyte is KCl solution with 1 Mol L⁻¹ during the experiment.) b–g) shows the curve of the current for λ -DNA testing experiments using single and dual nanopore chips. b) In the absence of λ -DNA, the variation of current was measured using a single nanopore. c) The variation of the current was measured using a single nanopore in the presence of 5.4 ug mL⁻¹ of λ -DNA. d) The detailed image and translocation model of λ -DNA translocation for the single-nanopore chip. e) In the absence of λ -DNA, the variation of the current was measured using a dual-nanopore chip. f) The variation of the current was measured using a dual-nanopore chip. f) The variation of the current was measured using a dual-nanopore chip. f) The variation of the current was measured using a dual-nanopore chip. f) The variation of the current was measured using a dual-nanopore chip. f) The variation of the current was measured using a dual-nanopore chip. f) The variation of the current was measured using a dual-nanopore chip. The arrows in (d) and (g) indicate the direction of DNA movement.

number during nanopore fabrication.^[8] However, the operation process is relatively complex.

Here, we focus on the problem of distinguishing the number of nanopores. A method for judging whether a chip has a single pore is proposed by analyzing the conductance composition and influencing factors. Based on this method, it is possible to easily and quickly identify whether a chip has a single pore. It facilitates the rapid screening of the chip with a single pore from those chips fabricated by the controlled dielectric breakdown method. It is expected to promote the practical application of the nanopore.

2. Experimental Section

The experiment includes three processes to judge the number of nanopores, including the preparation of the nanopore, the conductance testing, and the verification of the corresponding theoretical model.

The nanopores used in this study were prepared based on TEM (JEM 2100F, Accelerating voltage: 200 kV, emission current: 254 μ A) to ensure that the chips have a given number and diameter of the nanopore. A silicon nitride (SiN_x) thin film with a thickness of 30 nm (purchased from Norcada (#NT005X)) was

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Figure 2. Fabricated nanopore chips with different diameters and numbers using TEM (the scale bar is 20 nm).

used as a support membrane. Chips with different diameters and numbers of the nanopore were fabricated by TEM, as shown in **Figure 2.** The TEM images of all the nanopores used in the experiment are shown in Figure S2 (Supporting Information). The diameter of the prepared nanopores is in the range of 4–50 nm. Before the experiment, the chip was treated with oxygen plasma (instrument: PE-25 (Plasma Etch, Inc), vacuum: 200 mT, gas flow: 15cc min⁻¹, power: 70 mW, time: 2 min), and then immersed in IPA/deionized water (DIW) (1:1). Then, IPA/DIW was replaced by the KCl solution. The electrolyte was prepared using ultrapure water with PH 7.0. Finally, the chip was fixed in a self-made Faraday shielding box, and the conductance of the nanopore was tested using an Axopatch 200B.

3. Results and Discussion

3.1. Theoretical Analysis of the Nanopore

In addition to directly observing the diameter of the nanopore by conventional methods (such as TEM or scanning electron microscope (SEM)), measuring the conductance of a single pore can be used to determine the diameter. Before analyzing the number of nanopores determined by conductance, it is necessary to clarify the relationship between conductance and the diameter of the nanopore.

Many theoretical models for the relationship between conductance and diameter have been studied.^[9] The theoretical model generally decomposes the total conductance into three parts:^[10] bulk conductance G_{Bulk} , surface conductance G_{Surface} , and access conductance G_{Access} (= $1/R_{\text{Access}}$).

Conductance models constructed by Liebes et al.^[9a] and Frament et al.^[9b] consider the shape of the nanopore without considering access conductance. Access conductance based on the Hall model^[11] only considers the contribution from the nanopore region and does not consider the contribution of the surface charge. The conductance of nanopore together with the Hall model has the following form^[10]

$$G = \frac{1}{\frac{1}{G_{\text{Bulk}} + G_{\text{Surface,p}}} + R_{\text{Access}}}$$
(1)

Here, μ_K and μ_{Cl} represent the mobility of ion K⁺ and Cl⁻, respectively, *c* is the concentration of the KCl solution, ρ represents the resistivity of the KCl solution, *l* is the thickness of the nanopore, *r* is the radius of the nanopore, σ is the surface charge density of the nanopore, $G_{\text{Surface,p}} = \frac{2\pi r \mu_K |\sigma|}{l}$ means the surface

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Figure 3. Ionic flux density difference of the nanopore with and without surface charge. a) Structure of the nanopore on 2D space; b) Ionic flux density difference on the *r*-axis; c) Ionic flux density difference on the *z*-axis; d) Difference of the ion flux density vector with and without surface charge simulated based on Poisson-Nernst–Planck equations.

conductance inside the nanopore, $G_{\text{Bulk}} = \frac{\pi r^2}{l} (\mu_K + \mu_{Cl})Fc$, and $R_{\text{Access}} = \frac{\rho}{2r}$.

Much research has studied the contribution of the effect of the surface charge on the outer surface of the pore, but the entrance section of the nanopore is still regarded as an equipotential surface (we call it the serial circuit model).^[12]

$$G = \frac{1}{\frac{1}{\frac{1}{G_{\text{Bulk}} + G_{\text{Surface,p}}} + 1/\left(\frac{1}{R_{\text{Access}}} + \frac{1}{R_{\text{Surface,s}}}\right)}}$$
(2)

Here, based on the theory of Bocquet about the expression of the modified access conductance,^[12a] we separate the contribution of surface conductance from access conductance. $R_{\text{Surface},s}$ (= $1/G_{\text{Surface},s}$) means the surface resistance outside the nanopore and is expressed as follows:

$$R_{\rm Surface,s} = \frac{2\rho}{\beta L_{\rm Du}} \tag{3}$$

Here, $\beta = 2$ is the free parameter, L_{Du} is the Dukhin length and expressed as $L_{\text{Du}} = (|\sigma|/e)/(2c)$, *e* is the elementary charge.

Equation (2) is different from the actual situation because the surface charge will affect the potential near the surface. To analyze the effect of the external surface charge on the surface current, we performed a simulation analysis of the ion flux density distribution of the nanopore with the Freefem++ package (see http://www.freefem.org//ff++). The detailed simulation process can be found in the supporting information. Figure 3 shows the simulation results. Figure 3a shows the structure of the nanopore. Here, column coordinates are chosen for the coordinate system. Boundary 8 is the symmetry axis of the nanopore, and boundary 4 is the inner surface of the nanopore. Figure 3b shows the differential value in the total ion flux density (in the rdirection) between the two cases with and without surface charge. Figure 3c shows the differential value in the total ion flux density (in the z-direction) between the two cases with and without surface charge. In detail, to get Figure 3b,c we first get the flux

density under the condition with surface charge, and then subtract the flux density under the condition without surface charge. Figure 3d shows the differential value in the total ion flux density vector between the two cases with and without surface charge. We found that the differential value between the total ion flux density of the nanopore with and without surface charge is mainly in the inner and outer surface of the nanopore. So the equipotential surface assumption is canceled here. In this model, we attribute the current caused by the surface charge inside the pore to the contribution of the surface charge outside the pore. The specific circuit model is shown in Figure 4a. The conductive circuit model of the nanopore with surface charge is decomposed into two parts: resistance independent of the surface charge (including $R_{Surface s}$ and $R_{\text{Surface},p}$) and resistance introduced by the surface charge (including R_{Access} and R_{Bulk}). Here, $R_{Surface,s}$ represents the resistance introduced by the surface charge outside the nanopore, $R_{\text{Surface,p}}$ represents the resistance introduced by the surface charge inside the nanopore, and R_{Bulk} represents the resistance introduced by the bulk charge inside the nanopore.

Here, the theoretical model we use will consider all the contributions of four parts of the conductance, which has a similar form as graphene-based nanopore (we call it the parallel connection model)^[13] and is specifically expressed as follows:

$$G = \frac{1}{\frac{1}{G_{\text{Bulk}}} + R_{\text{Access}}} + \frac{1}{\frac{1}{G_{\text{Surface,p}}} + R_{\text{Surface,s}}}$$
(4)

To verify the validity of the model, we compared the experimental conductance (with chip No.1–5 as shown in Figure S2, Supporting Information) with the calculated result, as shown in Figure 4b. It can be found that the experimental data and the calculating result fit very well when the surface charge density is assumed to be -60 mC m^{-2} .^[14] Also, we compared the experimental data with the calculated results of the conventional series circuit model. We added detailed comparing deviation computed using $\sum_{i} \left(\frac{G_{i,eep}-G_{i,model}}{G_{i,eep}} \right)^{2}$. By comparing the deviation between Bocquet's model (0.5233) and our modified model (0.3307), we found



Figure 4. Nanopore conductance model and its comparison with experiments. a) Structure and circuit model of the nanopore in the calculation; b) Comparison between experiment and model with different surface charge density.

that our model has a lower deviation than Bocquet's model. Hall model is 0.7246. Deviations under only two concentration conditions of 1 and 0.1 Mol L^{-1} for Bocquet's model, our modified model, and Hall's model are 0.0170, 0.0044, and 0.0064, respectively. The modified model still shows the best fitting. And the parallel circuit model we used has a much smaller deviation. Therefore, we chose the parallel circuit model to solve the subsequent conductance.

3.2. Experimental Result of Single and Multiple Nanopores

The value of the conductance depends on the concentration and radius. Since there is a product term for the two parameters (concentration and radius) in Equation (4), the change in conductance caused by the concentration is related to the coefficient corresponding to the concentration term (containing the radius parameter). If the coefficients are different, the change in conductance due to a change in concentration may be different. Based on the above considerations, it is possible to identify whether a chip has a single pore according to the difference in conductance at different concentrations under certain conditions. For example, when a single nanopore chip (diameter = 20 nm, thickness = 30 nm) and a dual nanopore chip have the same pore conductance (106.1 nS based on Equation (4)) in 1 Mol L^{-1} KCl solution, the conductance in 0.1 Mol L⁻¹ KCl solution is different (13.43 nS for single pore and 15.43 nS for dual pore). Based on this, we model the conductance curves of different nanopores in 1 and 0.1 Mol L⁻¹ of KCl solution. Then the actual conductance of the nanopore in 1 and 0.1 Mol L⁻¹ of KCl solution was mapped onto the model curve to determine the number of nanopores.

When there are multiple nanopores on one chip, the conductance of the chip is obtained by summing the conductance of each pore. The conductance of multiple nanopores can be expressed as

$$G_{\text{device}} = \sum_{i=1}^{n} G_i = \sum_{i=1}^{n} \left(\frac{1}{\frac{1}{G_{\text{Bulk},i}} + R_{\text{Access},i}} + \frac{1}{\frac{1}{G_{\text{Surface},p,i}} + R_{\text{Surface},s,i}} \right)$$
(5)

Here, n(=1, 2, 3) represents the pore number in the chip. For the convenience of calculation, we assume that each nanopore in the multiple pores is equal in radius to each other. Therefore, the following equation holds

$$G_{\text{device}} = nG_{\text{i}} \tag{6}$$

To examine the validity of the model, we compared the experimental results and the conductance curves calculated by Equation (6) for chips with a single nanopore (chips from No.1-1 to No.1–5), two nanopores (chips from No.2-1 to No.1-3), and three nanopores (chip No.3-1) as shown in **Figure 5**. Here, the conductance of the nanopores was measured at concentrations of 1 and 0.1 Mol L^{-1} , respectively.

Figure 5 shows that the experimental results of the conductance of the single-pore chip are in good agreement with the conductance curves calculated by the model. To further validate the effectiveness of the model, we computed the overall deviation of our modified model and Bocquet's model based on



Figure 5. Calculation and experience of the relationship between the pore number and conductance.

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 $\sum_{i} \left| \frac{G_{i,exp} - G_{i,model}}{G_{i,exp}} \right|$. Here, *i* means the ith chip in the experiment. The overall deviation of our model is 0.2947, and that of Bocquet's model is 0.7737. This indicates that the modified model is effective. It shows that this method can distinguish whether the chip has a single pore, but it is difficult to determine the specific number of pores (specifically, two or three pores). We have analyzed the statistical deviation by computing $\left| \frac{G_{1,0,1M} - G_{x,0,1M}}{G_{1,0,1M}} \right|_{G_{1,1M} = G_{x,1M}}$ for curves in Figure 5, with x representing two and three pores represented by the second statement.

spectively. Here, $G_{1,0.1M}$ means conductance of a single nanopore at 0.1 Mol L⁻¹ solution, $G_{1,1M}$ means conductance of a single nanopore at 1 Mol L⁻¹. We calculated the maximum deviation between the experimental data and the model. (As shown in Table S2, Supporting Information) Among them, the maximum deviation between the experimental data of single pore devices and the single pore model is 5.47%; The minimum deviation between the experimental data of the dual pores devices and the single pore model is 12.24%; The deviation between the experimental data of the three pores devices and the single pore is 28.99%. So, if a set of experimental data deviates more than 5.47% from the single pore model, there is a possibility that it has more than one pore. If it exceeds 12.24%, it can be determined as multiple nanopores.

3.3. Factors Affecting the Accuracy of Predictions

To analyze the reasons for the inconsistency between the experimental data and the theoretical model for the chip with two or three pores, we studied the contribution of the difference between the model parameters and the actual parameters of the nanopore. These differences include the non-standard circular shape of the nanopore the inconsistency of the diameter of the prepared chip with two nanopores, the finite distance between multiple nanopores, as well as other possible factors.

3.3.1. Nonstandard Circular Shape of the Nanopore

Due to the randomness in the processing process, it is hard to ensure that the processed nanopores are standard cylindrical. More often, it looks like an elliptical column. Since the ellipse has a larger specific surface area than the circle, the surface charge of the ellipse contributes more to the pore conductance, which will inevitably affect the conductance distribution of the nanopore at different concentrations. Therefore, it is necessary to analyze the distribution law of the conductance curve when the aperture is not a standard cylinder. To facilitate the calculation of the conductance, we assume that the irregular nanopores have an elliptical columnar structure. Based on this, the perimeter (L) of the ellipse is expressed as^[15]

$$L = 4a \int_0^{\pi/2} \sqrt{1 - \frac{a^2 - b^2}{a^2} \sin^2 \theta} d\theta$$
 (7)

Here, *a* represents the long side of the ellipse, and *b* represents the short side of the ellipse. The area (*S*) of the ellipse is expressed as

$$S = \pi a b \tag{8}$$



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Figure 6. The effect of structural differences of single nanopores on the conductance, with enlarged views of some of the conductance curves shown in the insert.

Based on the capacitance calculated by Yang et al.,^[16] the access resistance of the nanopore is denoted as^[11]

$$R_{\text{Access}} = \frac{2\epsilon\rho}{C_{\text{cap}}} = \frac{\epsilon\rho}{\pi a\epsilon/K(k_0)} = \frac{\rho K(k_0)}{\pi a}$$
$$= \frac{\rho}{\pi a} \int_0^{\pi/2} \frac{d\theta}{\sqrt{1 - (k_0 \sin \theta)^2}}$$
(9)

With

$$k_0 = \sqrt{1 - \left(\frac{b}{a}\right)^2} \tag{10}$$

Here, ϵ represents the dielectric constant of the KCl solution, and C_{cap} is the capacity between the ellipse and infinity.

By changing the ratio of the long and short sides of the ellipse, we analyzed the effect of the shape on the conductance of a single nanopore. Figure 6 shows that the conductance curve of the irregular nanopore moves towards the direction of the conductance curve of chips with two pores as the ratio of the long and short sides becomes bigger. It is consistent with the intuitive impression that an ellipse has a larger specific surface area than a circle.

Most of the nanopores prepared in the experiment are not strictly circular but have a certain tendency to be elliptical. Therefore, the conductance of the actual chip is slightly higher than that of the ideal circular chip. But, even if the ratio of the long and short sides reaches 1:0.5, the tendency of the conductance to deviate from the conductance curve of a single cylindrical pore increased less than 4.5%, which is obtained by calculating $|\frac{G_{10.1M}-G_{ellipse.0.1M}}{G_{10.1M}}|$ for both chips having the same conductance at 1 Mol L⁻¹ solution. Generally, it is difficult for the prepared nanopores to achieve such a high ratio. Therefore, misjudgment is not easy to occur.





Figure 7. Conductance curves of multiple nanopores with unequal diameters to each other, with enlarged views of some of the conductance curves shown in the insert.

3.3.2. Inconsistent Diameter in the Case of Multiple Nanopores

During the preparation of the nanopore, it is difficult to ensure that the prepared nanopores have the same diameter, so there is a slight difference in the diameter between the chips with two pores and three pores (as shown in Figure 2). The diameter difference between the pores affects the total conductance distribution of the chip. So, Equation (5) is more accurate than Equation (6) in calculating the conductance distribution of the nanopore. Here, we take the chip with two pores as an example and calculate the conductance changes caused by the diameter difference using Equation (5). In the calculation, the aperture ratio between the big and the small pores in the double pores decreases from 1:1 to 1:0.1. **Figure 7** shows the calculated and experimental result.

The calculation result shows that when the aperture ratio increases, the conductance curve corresponding to two pores monotonically decreases in the direction of the conductance curve corresponding to the single pore. Figure 7 shows that the difference in diameter affects the conductance distribution at different concentrations. The diameter difference may cause the actual conductance curve to depart from the calculated curve. However, it is also clear from Figure 7 that the tendency to move significantly closer to the single-pore conductance curve only occurs when the aperture ratio is large (e.g., bigger than 1:0.5). In models with different diameters, when the diameter ratio reaches 1:0.3, the deviation of the model from the ideal dual pore model is smaller than the deviation from the single pore model, indicating that it can still be distinguished as dual pores; When the diameter ratio reaches 1:0.1, the deviation of the model from the ideal dual pore model is smaller than the deviation from the single pore model, indicating misjudgment. Further calculation shows that when the aperture reaches 1:0.21, it gets indistinguishable.

3.3.3. Finite Distance Between Multiple Nanopores

The conductance of multiple nanopores is affected by the porepore interaction,^[17] which may affect the conductance curve at different concentrations. Based on the theory of the pore-pore



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Figure 8. Conductance curves of multiple nanopores with different distances to each other, with enlarged views of some of the conductance curves shown in the insert.

interaction, we added the interaction term to our model. The total conductivity is represented as

$$G_{\text{device}} = \sum_{i=1}^{n} \left(\frac{1}{\frac{1}{\frac{1}{G_{\text{Bulk},i} + R_{\text{Access},i}} + \frac{1}{G_{\text{Surface},p,i} + R_{\text{Surface},s,i}}} + \frac{\rho \gamma}{2d_{pp}}} \right)$$
(11)

Here, $\gamma = n_{\text{pore}}^{0.5}$ is the coupling factor, d_{pp} represents the distance between the centers of two pores.

We compare the conductance of multiple nanopores under different pore-pore distances in Figure 8. To intuitively analyze the influence of spacing between pores, the distance represented in Figure 8 is the distance $(d_{pp} - 2r)$ between the edges of two pores. From the calculation result, we found that the pore-pore distance may cause the curve to shift downwards when the distance gets near to each other. As the distance decreases, the deviation increases. By computing the deviation us- $\log |_{\frac{G_{1,0.1M}-G_{2dis,0.1M}}{-}}$ and $\left|\frac{G_{1,0.1M}-G_{2,0.1M}}{G_{1,0.1M}}\right|$, we found $G_{1.0.1M}$ $G_{1,1M} = G_{2,1M}$ $G_{1,1M} = G_{x,1M}$ that, for double pores with a spacing of 25 nm between each other, the deviation increases with the increase of nanopore diameter. (As shown in Figure S3, Supporting Information) When the diameter of each pore reaches 40.6 nm, the deviation from the ideal dual pore model is equal to the deviation from the single pore model, resulting in misjudgment.

There may also be a problem during the experiment that the prepared nanopores have different opening angles. Since such experiments are difficult to verify, this case is not analyzed here.

4. Conclusion

In summary, to overcome the challenges of using nanopores for precise measurements, we investigated an approach that can easily and quickly distinguish whether a chip has a single pore or not and analyzed several factors that affect the basis of judgment.

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The results show that by separately testing the conductance of the nanopore at two different concentrations and comparing it with the conductance curve obtained from the theoretical model in the case of a single pore, it is possible to identify whether the tested chip has a single pore. Additionally, we delve into various factors that influence the conductance curve, such as deviations in pore shape from the standard circle, inconsistencies in pore diameter, and the finite distance between multiple nanopores. Since the shape of the prepared nanopores is not always a standard circle, we use an ellipse to approximate the actual nanopore shape here. In this approximation, the calculated conductance curve for the elliptical pore shifts in the direction of the conductance curve of multiple pores when the ratio of the long and short sides increases. Since the actual nanopore does not have a large ratio of the long and short sides, the change in the conductance is not obvious. So it does not affect the judgment. Due to the difference between the diameters in the case of double or multiple pores, the conductance of multiple pores obtained in the experiment deviates from the conductance curve obtained by the model. So it is difficult to distinguish the specific number of multiple pores. At the same time, the finite distance between multiple nanopores can cause the curve to shift downward when the distance gets near to each other. When the pore-pore distance is too close and the aperture is too large, misjudgment may occur. This approach significantly enhances the practical application of low-cost nanopore preparation techniques and can easily and effectively achieve the judgment of whether a chip has a single nanopore, which is a guideline for the judgment of the number of nanopores prepared by controlled dielectric breakdown and other methods.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

access resistance, pore number, solid-state nanopore, surface charge, the shape of the nanopore

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