High-Throughput Nanocapillary Filling Enabled by Microwave Radiation for Scanning Ion Conductance Microscopy Imaging

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ABSTRACT: Solid-state nanopores provide a highly sensitive tool for single-molecule sensing and probing nanohydric effects in solutions. Glass nanopipettes are a cheap and robust type of solid-state nanopore produced from pulling glass capillaries with opening orifice diameters down to below tens of nanometers. Sub-50 nm nanocapillaries allow an unprecedented resolution for translocating single molecules or for scanning ion conductance microscopy imaging. Due to the small opening orifice diameters, such nanocapillaries are difficult to fill with solutions, compromising their advantages of low cost, availability, and experimental simplicity. We present a simple and cheap method to reliably fill nanocapillaries down to sub-10 nm diameters by microwave radiation heating. Using a large statistic of filled nanocapillaries, we determine the filling efficiency and physical principle of the filling process using sub-50 nm quartz nanocapillaries. Finally, we have used multiple nanocapillaries filled by our method for high-resolution scanning ion conductance microscopy imaging.

KEYWORDS: solid-state nanopores, glass nanopores, nanocapillaries, scanning ion conductance microscopy, fluid dynamics

INTRODUCTION

Solid-state nanopores are a promising new tool to study various biochemical interactions at the molecular scale. In order to perform sensing, an electric potential is applied through a small pore. The sensing is based on the localized conductance drop that depends on the ionic concentration, surface charge, volume, and shape of the pore. Solid-state nanopores tend to have higher current stability than their biological counterparts since biological nanochannels are limited by membrane stability, which can be affected and ruptured by electroporation at high bias voltages (200–300 mV). Silicon dioxide nanochannels are one of the most widely used platforms for various biosensing applications such as single protein detection, DNA folding sensing, DNA–protein interactions, etc. Besides biosensing applications from the bulk solutions, the sharp and highly conical geometry of silicon dioxide nanochannels is advantageous for various surface scanning applications and they are typically used for scanning ion conductance microscopy (SICM). Further developments of the SICM technique were used to probe surface charge and surface stiffness. Similarly, as for nanopore sensing, the main parameter that determines the resolution of SICM methods is the radius of a nanopore; therefore small nanochannels are preferably used. However, glass nanochannels under 50 nm are problematic to fill with aqueous solutions.

The common method to fill sub-50 nm nanochannels is the use of commercially available capillaries containing a glass filament. However, this alters the conical geometry of a nanochannel. Another method is to prefill the capillaries with a solution with a lower surface tension such as ethanol and then to exchange the solution back to an aqueous electrolyte. This exchange procedure is time-consuming, which depends on the length of the capillary tip, and can cause clogs or additional nonlinearities in the capillary conductance. Finally, ethanol to water exchanges foster the formation of nano-bubbles on contaminated or hydrophobic surfaces that can significantly increase ionic current rectification and induce measurement artefacts.

As a consequence, several nanochannel filling techniques have been developed to fill sub-50 nm nanochannels directly with the final solution of interest without prefilling steps. The first heating-based approach called dynamic microdistillation relies on selective heating of the nanochannel tip with heat applied from a conductive coil. Using this technique, sub-20...
Microwave status is indicated as either ON or OFF. A video of the filling process is provided as Video 1. We demonstrate a fast and high throughput method using microwave radiation-induced rapid heating of the solution in which nanocapillaries are immersed. As shown in the schematic in Figure 1, capillaries were first placed in a filling solution bath, glued on a glass slide for improved handling. Due to high surface physisorption affinity to water molecules after oxygen plasma treatment, aqueous solution filled the very tip of a 47 nm nanocapillary (Figure 1a) by immersing capillaries into solution. As the capillary is tapered, the liquid easily fills the narrow tip due to increased capillarity (Figure 1b) until Laplace pressure in equilibrium with the local pressure due to the local widening. This is explained by the Young–Laplace equation (eq 1)

$$P = \frac{2 \sigma_{gs} \cos \theta}{r}$$

where $\sigma_{gs}$ is the interfacial energy between gas and solution, $\theta$ is the wetting angle, and $r$ is the radius of a capillary.

Subsequently, a batch of nanocapillaries is placed in a desiccator and kept in 1–10 mbar absolute pressure to degas the solution and further prefill nanocapillaries. This enables to fill the thick end of the capillary with the solution while leaving a pocket of air near the tip (Figure 1b) and typically took a few minutes to complete. The formation of this air/water interface typically prevents nanocapillaries from immediate filling and nanocapillaries were successfully filled. However, this method has low throughput and requires precise handling of fabricated nanocapillaries. Alternatively, a microcentrifugation approach was also used to fill 30–200 nm wireless nanocapillary-based electrodes, which has similar limitations. Recently, a second thermally-driven approach has been shown to simplify the filling procedure and increase throughput to tens of nanocapillaries. The procedure is based on the fact that a temperature gradient between the tip and the body of the capillary varies the surface tension and drives the movement of small bubbles out of the capillary. This method requires keeping capillaries for tens of minutes at a high temperature, which is incompatible with solvents that evaporate and alter the conductivity of the buffer. The method demonstrated 100% filling efficiency based on brightfield microscopy images. Further electrical measurements are needed to evaluate the electrical signal of filled nanocapillaries by measuring conductance and rectification values.

This work aims to provide an accessible and robust filling method for glass nanocapillaries below the 100 nm size range. We demonstrate a fast and high throughput method using easily accessible microwave radiation, which is faster than methods published previously and has a 85.9% filling rate calculated based on electrical measurements, while optical inspection gives the 100% filling rate. We correlate the imaged size of these nanocapillaries with their electrical conductance and rectification factor to give additional insights into the resulting electrical signal quality. Furthermore, we apply the fabricated nanocapillaries for scanning ion conductance microscopy imaging and compare the image quality of different diameter nanocapillaries.

### RESULTS AND DISCUSSION

Our batch nanocapillary filling procedure is based on microwave radiation-induced rapid heating of the solution in which nanocapillaries are immersed. As shown in the schematic in Figure 1, capillaries were first placed in a filling solution bath, glued on a glass slide for improved handling. Due to high surface physisorption affinity to water molecules after oxygen plasma treatment, aqueous solution filled the very tip of a 47 nm nanocapillary (Figure 1a) by immersing capillaries into solution. As the capillary is tapered, the liquid easily fills the narrow tip due to increased capillarity ($P_c$) until Laplace pressure is in equilibrium with the local pressure due to the local widening. This is explained by the Young–Laplace equation (eq 1)

$$P_c = \frac{2 \sigma_{gs} \cos \theta}{r}$$

where $\sigma_{gs}$ is the interfacial energy between gas and solution, $\theta$ is the wetting angle, and $r$ is the radius of a capillary.

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Figure 1. Schematics and microscopy images of the method sequentially used for filling nanocapillaries. Brightfield microscopy images of the same capillary in different steps are displayed on the bottom left in each panel. The solution is false-colored in blue, and the scale bar in each image is 50 μm. (a) Schematics of a batch of nanocapillaries in the salt solution. (b) Schematics of a desiccator showing a gas bubble forming near the tip of the capillary marked with black arrows. (c) Schematics of the capillary under microwave radiation. The measured diameter of the nanocapillary displayed here is 47 nm.

Figure 2. Nanocapillary filling process dynamics. (a) Schematics of the main filling steps: (step 1) Capillaries are prefilled in the desiccator. (step 2) Microwave radiation is applied, thus causing rapid heating of the solution until it boils. This has the effect of reducing the size of the gas bubble. (step 3) Heating is applied in a few cycles, fully filling the nanocapillary in a matter of minutes. (b) Time-lapse snapshots of the filling process while filling 28 nm (top) and 26 nm (bottom) nanocapillaries. The time of each snapshot from the start of the recording is indicated in seconds, and the microwave status is indicated as either ON or OFF. A video of the filling process is provided as Video 1.
forming electrical contact, required for further measurements. In this study, we ensured the rapid heating of the solution by irradiating the capillaries placed in a solution bath with microwave radiation \( (\lambda = 12.2 \text{ cm}) \) (Figure 1c). Heating was performed in cycles by heating up the solution until its boiling point and letting it cool down for 10–20 s. Heating duration varied based on the volume of the solution used to immerse the nanocapillaries, and microwave radiation was always applied until the boiling point only to minimize evaporation. By performing the described procedure (3–5 cycles of heating and cooling), we were able to completely fill batches of sub-50 nm nanocapillaries in less than 10 min, out of which for most of the time (90%), the solution was not boiling. The length of the procedure varied in order to ensure the complete filling of the batch, but as shown in Figure 2, it can be reduced to less than 2 min, compared to 20 min, when using a thermally-driven approach.\(^1\)\(^9\) The batch size was limited by the number of pulled and characterized capillaries and typically was 25 but can be significantly increased for high-throughput fabrication pipelines.

To explain the mechanism behind the filling of the nanocapillaries, we studied the time evolution of this process (Figure 2) with real-time recording using a custom-built imaging setup (see the Experimental Section for more details). After partial filling of the fabricated capillaries in a desiccator (Figure 2a (step 1) and also Figure 2b marked as 0 s), the batch of nanocapillaries is heated with microwave radiation by placing in a microwave oven (Figure 2a, step 2). The microwave radiation induces rapid heating and subsequent boiling of the solution. According to Henry’s law, the solubility of gases is reduced with an increase in temperature. The microwave radiation causes not only overheating of the solution\(^2\)\(^0\) but also oversaturation of the liquid with gas, which then nucleates into bubbles. Subsequently, gas bubbles grow until they blow up violently, causing additional mixing of the solution inside and outside the capillary and improving transfer of gas outside the capillary. (Figure 2b, 0–40.4 s). In the process, the solution is degassed and the gas content in the solution inside the capillary is reduced (Figure 2b, 57.2 s). After the microwave heating is deactivated, the liquid cools down and starts to absorb the excess gas inside the capillary. Additional cycles (Figure 2b, 57.2–96.1 s) cause the same behavior. This is observed at the end of the process when only one or more small bubbles are attached to the sidewalls of the capillary (Figure 2b, 96.1–118.8 s). As the solution is cooling down, its gas capacity is also increasing which results in air bubbles being absorbed into the now undergassed solution until there is no visible obstructions (Figure 2b, 118.8 s). This behavior was observed in all filled capillaries, so we argue that the reason for the fact that microwave radiation is faster than classical heating methods (with filament-induced\(^1\)\(^7\) or hot plate-induced\(^1\)\(^9\) heating) is the high-intensity, localized super-heating of the liquid inside the capillary. This causes rapid and efficient overgassing of the liquid, which then transfers the excess gas outside. Microwave radiation is known as a method for enhancing reaction kinetics in both aqueous and non-aqueous solutions\(^2\)\(^1\) and for degassing.\(^2\)\(^1\) Corresponding IV curves are shown in Figure S1. In addition we have confirmed the ability to fill the capillaries with glycerol–water and agarose–water solutions by using this method (Figure S2). This shows that our method is applicable to a wide range of solutions regardless of high viscosity.

In order to test the efficiency of the filling method of microwave radiation-induced filling, multiple nanocapillaries were analyzed by measuring the size of the nanocapillaries with a scanning electron microscope, taking brightfield microscopy images before and after the microwave radiation-induced filling and measuring the electrical characteristics such as IV curves and noise levels (Figure S6) to quantify the electrical parameters after the filling procedure (Figure 3). Nanocapillaries of different sizes visually examined after the typical filling procedure showed 100% filling success rate. Similar results were previously reported with a thermally-driven approach.\(^1\)\(^9\) In this study, all the fabricated capillaries (\( N = 179 \)) were further electrically characterized by recording IV curves and 14.1% of the nanocapillaries displayed electrical characteristics of the nanocapillaries that were either mechanically damaged (broken with much larger current reading) or clogged (no electrical contact). This number could be elevated due to capillary braking and contamination not related to the filling procedure; however, we used it to estimate the filling success rate. The dependency of conductance on the diameter of the nanocapillaries as measured by SEM (scanning electron microscopy) (Figure 3a) was fitted with a conductance model adapted from a previously published study\(^2\)\(^2\) (eq 2).
with the protocol described in this paper. (a) Schematics of a SICM setup used for imaging. (b, c) Topographical image of a fixed COS-7 cell scanned with (b) 80 nm and (c) 30 nm nanocapillaries. (d) Corresponding zoomed-in images of the areas marked in (b) (top) and in (c) (bottom). (e) Line profiles from panel (d) indicated in green and blue.

\[ G = \sigma \left( \frac{4f}{\pi D} + \frac{1}{2D} + \frac{1}{2d} \right)^{-1} \] (2)

D is the shaft diameter (here 400 μm), d is the diameter of the nanopore, and t is the taper length, which was determined to be 2 mm in our experiments. The indicated fit follows a linear trend for a capillary size range of 10–100 nm. The intrinsic variation for the small diameter (<20 nm) nanocapillaries can be caused by multiple factors such as quality of SEM measurements of the capillary’s opening due to charging effects or a lack of a precise estimate of the three-dimensional geometry of the nanocapillary. Furthermore, an increase in the rectification factor with a decrease in nanocapillary diameter was observed (Figure 3b) in the nanocapillaries. This is in agreement with previous reports where smaller nanocapillaries are known to have a larger ionic current rectification factor.23

Finally, microwave filled nanocapillaries were used for scanning ion conductance microscopy imaging (Figure 4). The SICM was chosen as a proof of principle platform for filled nanocapillaries. Imaging was performed with a homebuilt scanning ion conductance microscope (Figure 4a). Briefly, the scanning ion conductance microscope was mounted over an inverted optical microscope (Olympus IX 73). The sample was scanned in X and Y using a piezo stage, and the capillary was used as a Z-actuator. Characterized 30 nm and 80 nm capillaries filled with 400 mM KCl solution (pH = 7.5) were used to image the membrane surface of fixed COS-7 cells. In particular, microvilli were imaged as a structure of interest to perform a comparison of SICM resolution by using different diameter nanocapillaries. Previously reported dimensions of this membrane structure are ~1000 nm in length and 100 nm in diameter.24 This corresponds well with the values measured with the 30 nm pipette. The theoretical lateral resolution of SICM is approximately three times the inner opening radius of the nanocapillary \( d_0 = 3d \).24 In Figure 4b, the microvilli cannot be distinguished clearly when imaged with a 80 nm nanocapillary (\( d_0 \approx 120 \) nm). However, a 30 nm (\( d_0 \approx 45 \) nm) nanocapillary allowed us to visualize the topography of microvilli with the expected topographical dimensions as stated previously.

## CONCLUSIONS

We have demonstrated an efficient and fast method to fill sub-100 nm quartz nanocapillaries by using microwave radiation-induced heating. We have experimentally evaluated the throughput of the method visually and by performing electrical measurements with fabricated and filled nanocapillaries. To demonstrate the practical application of the fabricated nanocapillaries, we have performed an SICM imaging experiment and qualitatively demonstrated the impact of capillary size for the imaging resolution. We believe that the nanocapillary filling method proposed in this paper can be useful for the nanofluidic devices requiring a large number of filled nanocapillaries with various aqueous solutions.

## EXPERIMENTAL SECTION

**Nanocapillary Fabrication and Characterization.** Nanocapillaries used in our experiments were fabricated using a CO2 laser puller (P-2000, Sutter Instrument). Quartz capillaries with 0.5 mm outer diameter and 0.2 mm inner diameter were bought from Hilgenberg GmbH. Before the pulling process, all capillaries were cleaned with 100% acetone, 100% ethanol, Milli-Q water (Millipore Corp.), and again with 100% ethanol by sonicating in each solution for at least 10 min. After washing, nanocapillaries were dried in a desiccator for 1–2 h until they were completely dry. The pulling program used to fabricate nanocapillaries is shown in Table S1 (Supporting Information). After the fabrication, nanocapillaries were characterized using a scanning electron microscope (Zeiss, Merlin). All nanocapillaries were fabricated using a laser pipette puller with a protocol optimized for the fabrication of nanocapillaries with a 30–50 nm diameter range. Nanocapillary diameters were confirmed by SEM, and as expected, under relatively high imaging current (400 pA), capillaries under 40 nm shrunk due to electron beam heating-induced effects. However, the shrinking process did not significantly alter nanopipette geometry as the size difference before and after shrinking has not exceeded half of the capillary diameter. Diameters of...
all nanocapillaries were measured manually based on SEM images using Fiji software, as it is shown in Figure S7.

**Solutions.** KCl solutions used in this study were prepared from Milli-Q water (18.2 MΩ cm at 25 °C, Millipore Corp.). KCl solutions (400 mM) were buffered with 40 mM TRIS by adjusting the pH to 7.5 with HCl using a pH meter. All solutions were filtered with 20 nm filters (Whatman Anotop 25 Plus) before use.

**Filling of Nanocapillaries.** After SEM imaging, nanocapillaries were placed on cover glass with double-sided polyimide (Kapton) tape. Nanocapillaries were then cleaned with oxygen plasma (Femto A, Diener electronic GmbH) for 600 s at maximum power setting. Immediately after, the nanocapillaries were immersed in a 400 mM KCl solution and placed inside the desiccator connected to a vacuum pump. Nanocapillaries were kept under low pressure conditions (1–10 mbar) for 10 min in order to prefill them. Then, they were imaged with an inverted brightfield microscope (Figure S4). After the prefilling step, the nanocapillaries were placed inside the microwave oven (MW 1766 EASY WAVE, P = 700 W, λ = 12.23 cm). The highest power setting was always used. Microwave radiation was applied in heating cycles in order to heat the solution until its boiling point. Heating duration varied based on the volume and temperature of a solution. The first heating phase took 30–60 s, and subsequent heating phases were significantly shorter (5–10 s) due to increased temperature of the capillary immersion solution. Heating was always applied until the boiling point of a solution in order to minimize the evaporation. Short 10–20 s pauses were made between heating steps (Video 1) to allow for the gas to dissolve in the solution. At least three heating cycles were performed to complete the filling of the batch. Nanocapillaries were imaged with a brightfield microscope. After the procedure, the buffer was exchanged to ensure that salt concentration was not affected by evaporation. For storage, nanocapillaries were kept at 4 °C.

**Brightfield Microscopy Imaging.** Nanocapillaries were imaged with a brightfield microscope, equipped with a 2.5X objective (Zeiss EC Plan, NA = 0.06) and sCMOS camera (Hamamatsu OrcaFlash 4). Nanocapillaries were imaged in a 400 mM KCl solution prepared as described previously. Basic image analysis and processing were performed in Python and Fiji.

**Recording of Nanocapillary Filling Dynamics.** The nanocapillary filling process was recorded with a long-focal distance USB microscope (Dino-light, AM3113T) mounted outside the microwave oven (WD700) at 2 P = 700 W, λ = 12.23 cm) using a white-light LED illumination source. The schematics of the imaging chamber used is displayed in Figure S3. Since all the microwave heating experiments were performed at the regular ambient pressure, the boiling point was determined visually by observing the solution and can be clearly seen in Video 1.

**Recording of IV Curves.** Current–voltage (IV) curves were measured with Ag/AgCl electrodes in a dedicated Teflon pipette holder filled with 400 mM KCl, placed in a Faraday cage to reduce noise from external sources (Figure S5). The current generated was amplified with a Femto DLPCA-200 (100MΩ gain) at 7 kHz. The data was recorded with a dedicated LabVIEW (National Instruments) software. Interface by sweeping the bias voltage from −500 to 500 mV in 10 mV gradual steps, thus spending the same time in each interval. IV current traces were analyzed and plotted with a custom-written Python script.

**Preparation of a Fixed COS-7 Cell Sample.** #1.5 cover glass coverslips were cleaned with a piranha solution and coated with fibronectin from bovine plasma (0.5 μM/mL). African green monkey kidney fibroblast-like cells (COS-7), purchased from ATCC, were grown in DMEM without phenol red medium, containing 10% of fetal bovine serum. Then, cells were fixed with 4% PFA with 0.02% Triton X-100 in 1xPBS (pH = 7.4) for 15 min and washed thrice for 5 min each with PBS (pH = 7.4). Imaging was performed in 400 mM KCl (pH = 7.5) solution. All chemicals were purchased from Sigma Aldrich, unless stated differently.

**Scanning Ion Conductance Microscopy.** Scanning was performed with a custom-made SICM setup. The sample was actuated in X and Y using a piezo stage (Piezo system Jena TRITOR102SG). The capillary was moved in Z using a homebuilt actuator, operated in hopping mode. The hopping height was 1 μm at a 100 Hz rate. The current set point used in the hopping actuation was 99% of the current recorded. Images with 512 × 512 pixels were generated with a pixel size of 39 nm.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsanm.0c01345.

- Nanocapillary pulling parameters (Table S1), nanocapillary filling dynamics and IV curves of filmed nanocapillaries (Figure S1), nanocapillary filling with high-viscosity solutions (Figure S2), schematics of a chamber used for direct imaging of the nanocapillary filling process (Figure S3), brightfield microscopy characterization of nanocapillaries before and after microwave-induced filling (Figure S4), schematics of the electrical signal measurement setup (Figure S5), RMS noise and power spectral densities of measured nanocapillaries (Figure S6), and SEM images of the nanocapillaries and corresponding IV curves (Figure S7)
- Video of filling of capillaries (MP4)
- Video of filling with 50% glycerol 400 mM KCl solution (MP4)

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**Notes**

The authors declare no competing financial interest.

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