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 nanolithic 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 nanopores are limited by membrane stability, which can be affected and ruptured by electroporation at high bias voltages (200–300 mV). Silicon dioxide nanocapillaries 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 nanocapillaries 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 nanocapillaries are preferably used. However, glass nanocapillaries under 50 nm are problematic to fill with aqueous solutions.

The common method to fill sub-50 nm nanocapillaries is the use of commercially available capillaries containing a glass filament. However, this alters the conical geometry of a nanocapillary. 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 nanobubbles on contaminated or hydrophobic surfaces that can significantly increase ionic current rectification and induce measurement artefacts.

As a consequence, several nanocapillary filling techniques have been developed to fill sub-50 nm nanocapillaries directly with the final solution of interest without prefilling steps. The first heating-based approach called dynamic microdistillation relies on selective heating of the nanocapillary 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 heating process is provided as Video 1. 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.

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 2). This method demonstrated 100% filling efficiency based on brightfield microscopy images. Further electrical measurements are needed to evaluate the electrical signal quality 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 (Pc) 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.

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...
In this study, we ensured the rapid heating of the solution by irradiating the capillaries placed in a solution bath with microwave radiation ($\lambda = 12.2$ 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. 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 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 degassed 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 or hot plate-induced 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 and for degassing. 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. 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 (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.

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

(2)

\(D\) is the shaft diameter (here 400 \(\mu m\)), \(d\) 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.

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 moved in Z using a homebuilt 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 \(\sim\)1000 nm in length and 100 nm in diameter. 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 \(d_0 = 3d\). In Figure 4b, the microvilli cannot be distinguished clearly when imaged with a 80 nm nanocapillary \((d_0 \approx 120 \text{ nm})\). However, a 30 nm \((d_0 \approx 45 \text{ nm})\) 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 CO\(_2\) 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.
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 polymide (Kapton) tape. Nanocapillaries were then cleaned with oxygen plasma (Femto A, Diener electronic GmbH) for 660 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, A, Diener electronic GmbH) for 660 s at maximum power setting.

**Nano-carr.** 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 phases were signified by evaporation. Short 10−20 s pauses were made between heating steps and

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**ASSOCIATED CONTENT**

**Supporting Information**

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

**Video of filling of nanocapillaries (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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