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Kwong-Li Probes: Novel Nano-Probes for Biological Dissection and Injection

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Abstract

We recently discovered a very fast and reliable single-step etching process to fabricate tiny-angled (<2.7°) fiber probe tips, which is less complex and yields finer tips than other available multiple-step sharp-tip (e.g., SNOM tips) fabrication processes. The process is named as Kwong-Li's (KL) Method. As the probe profile depends highly on the interfacial meniscus of the etchant, by means of our sacrificial boundary etching technique, in which we introduced glass tubings as etching barriers, probes with very sharp tips and long tapers were formed. Using p-xylene as organic solvent and hydrofluoric acid as etchant, we succeeded in shaping optical fibers (with initial fiber diameter of 125μm) into sharp tips with angles ranging from <2.7 – 9.7°, with nanoscale tip diameter of <1μm. By adjusting the initial etchant height in the tubing, final tip angles can be controlled. With their nanometric tips, these sharp probes will be useful for various scanning probe microscopy applications and could potentially be used as surgical tools for micro cellular surgery, i.e., we have already shown that KL probes will penetrate through cell membranes with less mechanical resistance than conventional pipettes and probes made by Turner's Method. The fabrication process of KL probes and experimental results from using KL probes to probe cells are presented in this paper.

Keywords

Sacrificial Boundary Etching; SNOM Tips; Nano Tips; Kwong-Li Probes; Kowng-Li Method

INTRODUCTION

Interfacing with the Nano-World is no longer a scenario in science fictions. Examples of nano sensing and manipulation are everywhere, such as DNA/cell detections in medical sciences, and nanotube manipulation to build novel electronic devices and sensors. These increasing needs in nanometric characterization, analysis and manipulation have triggered a thirst for a real-time nanometric imaging tool. Traditional optical microscopy is a real-time imaging technique. However, its spatial resolution is limited to one-half of the employed light wavelength [1], which is manifestly insufficient for current research requirements in the Nano-World. Although existing microscopy techniques, such as Scanning Tunneling Microscopy (STM), and Atomic Force Microscopy (AFM), can measure down to 10nm copy (AFM), can measure down to 10nm spatial resolution easily [2], their imaging principles are unfortunately not real-time, and this implies dynamic samples, which are common in life sciences research, cannot be handled by any of them.

In 1984, Scanning Near-field Optical Microscopy (SNOM) was invented to allow potential optical imaging in the nanometer scale. Its spatial resolution can go down to sub-100nm [2], which is far beyond the classical optical microscopy diffraction limit. This promising technique not only has a nanometric resolution, but also retains useful contrast mechanisms in traditional optical microscopy, e.g., polarization and fluorescence [3].

Like all other scanning probe microscopies, probe profile characterizes the resolution of SNOM. In general, the smaller the probe tip diameter, the better the resolution. And by intuition, the sharper the tip, the smaller the tip angle [4]. Currently SNOM fiber probes are produced by mechanical pulling and chemical etching [3]. Among these, chemically etched probes have a higher optical transmission. Yet, there are limited mechanisms which can easily realize a large range of fabricated probe angles with such high optical transmission.

In this paper, we present a novel probe fabrication process named as Kwong-Li's (KL) Method, in which a *sacrificial boundary etching* technique was introduced to a well known and simple chemical etching process (Turner's Method [5]). By controlling the initial etchant height in the sacrificial barrier, final tip profiles were demonstrated to be adjustable. Typical characteristics of these KL probes, such as probe profile, tip diameter and probe angle are discussed in this paper.

PRINCIPLE OF KL METHOD

In Turner's Method, pure Hydrofluoric (HF) acid is employed as etchant, while an organic solvent layer is used to cover it, resulting in an interfacial meniscus. The stripped fiber is then dipped into the etchant and the final probe tip is formed at the interfacial meniscus, as shown in Figure 1.

Such probe profile can be modeled by a solution of the Young-Laplace Equation [3], which reveals the relationship between the changes of interfacial meniscus to the final probe profile. Inspired by this discovery, KL Method introduces glass tubing as the sacrificial barrier to Turner's Method to etch the stripped fiber. During etching, HF continues to etch away the inner wall of the glass tubing, so the interfacial meniscus keeps falling, which is illustrated in Figure 2. As a result, the final probe formed has a long taper and a pointed tip. To control the probe profile, we can simply adjust initial HF height (h) in the glass tubing as defined in Figure 2.

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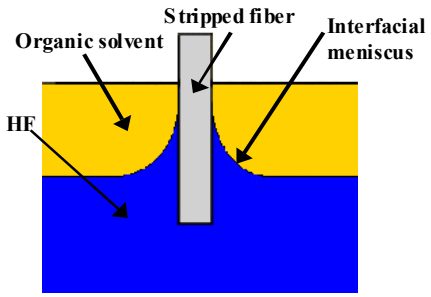

Figure 1. Illustration of Turner's Method.

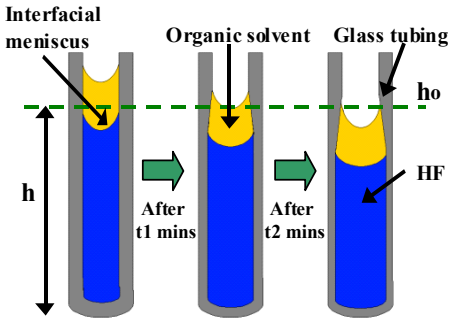

Figure 2. Controlling the interfacial meniscus by sacrificial boundary etching.

EXPERIMENTAL RESULTS

A set of sacrificial boundary etching experiments was conducted with glass tubings of 21mm inner diameter. Fiber probes were also fabricated by Turner's Method as a control to KL probes. In all experiments, single mode fiber was used (F-SA, Newport Corporation), with cladding diameter of 125µm. Etchant and organic solvent in the experiments were pure HF acid (48%) and p-xylene. These experiments were carried out in 16.5˚C environment, where the typical time for the process was 55 minutes. A KL probe fabricated using the above process is compared with a Turner's probe and an AFM tip as shown in Figure 3. The frontal views of a KL probe, a Turner Probe, and a pulled glass pipette is shown in Figure 4. As clearly shown in these Figures, KL probes have much smaller tip angle and longer taper than the conventional Turner probes used for SNOM. Intuitively, this implies that KL probes can be used to probe or dissect biological cells with much less mechanical resistance on the cell membranes. On the other hand, KL probes will also allow biologists to conduct experiments with much smaller cells than using mechanically pulled probes or Turner probes.

Probe profile

Typical KL probes have long tapers and very sharp angles as shown in Figure 5. Taper of fabricated KL probe can be longer than 2mm (see KL probe 3), which is much longer than a typical Turner probe, whose length is just 332.5µm as limited by Turner's process. The vast difference in the profiles of KL probes (1 – 3) and Turner's probe is also indicated in Figure 5. Again, the enormous difference between KL and Turner probe tips is obviously seen in Figure 5. Using the p-xylene, typical tip diameter of Turner probe was about 1µm. For KL probe tips, diameters ranged from 500nm to 1.5µm can be obtained. Typical KL probe tips are shown in Figure 6. Difficulty in determining the exact etching time may account for the deviations in probe diameters and shape, as HF may continuously diffuse up into the p-xylene layer which causes the very sharp tip being etched away.

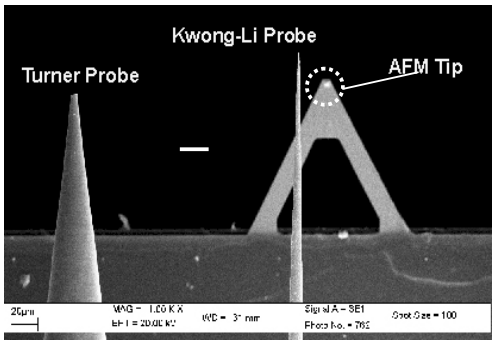

Figure 3. SEM picture of KL probes and Turner's probe. The scale bar is 20µm.

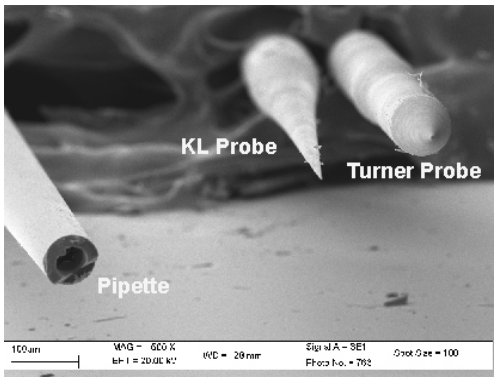

Figure 4. SEM picture of a KL Probe, a Turner Probe, and a conventional pipette. The scale bar is 100µm.

Probe angle

One of the greatest advantages of the KL Method is that the final tip angle can be sharpened down to <2.7˚ (sharpest angle reported to-date for optical fibers by chemical etching, to the best to the authors' knowledge). For instance, to form a sharper angle, we can simply increase *h*, where *h* is the initial HF height defined as in Figure 2. Experiments were carried out to validate this. A plot of KL probe tip angle versus *h* is given in Figure 7.

CELL PROBING EXPERIMENTS

A micromanipulation station with a µN sensing system was developed in our prior work [6] to detect force during the micro probing/injection process. The sensing system consists of a PVDF sensor and adapters to a micromanipulator and a probe. We have previously shown that our PVDF sensor can resolve micro-Newton force within a proper frequency range (resonance frequency at ~50Hz and detectable frequency at a few mHz).

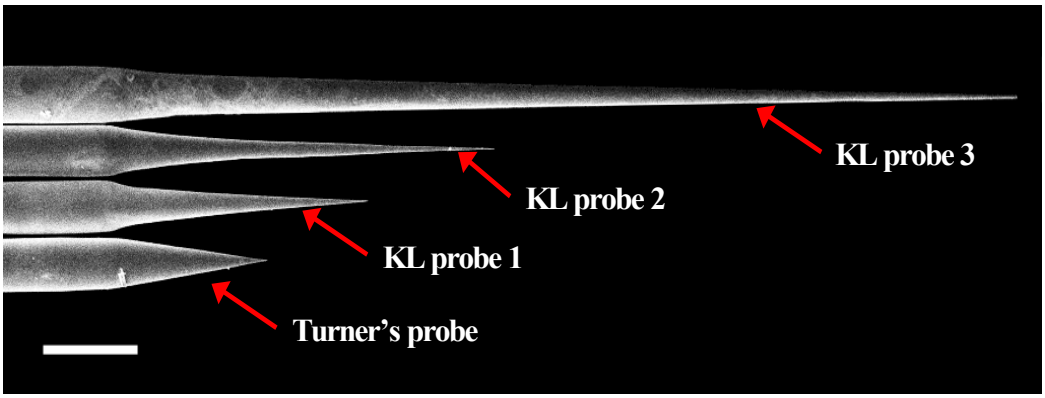

Figure 5. SEM picture typical KL probe tips compared to a Turner Probe. The scale bar is 200μm.

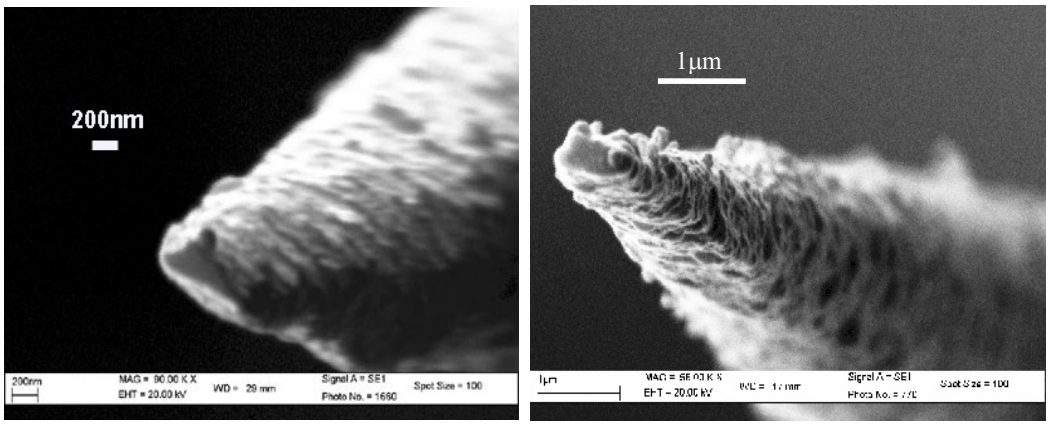

Figure 6. SEM pictures of typical KL probe tips. The tip profiles can be controlled by time in etchant.

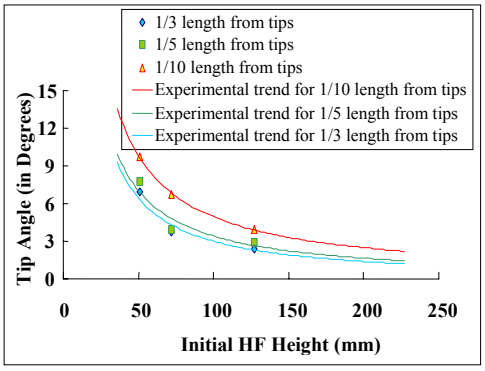

Figure 7. Relationship between KL probe tip angles and initial HF height.

In the currently work, we attached the Turner and KL probes to the sensing system to probe and penetrate cells under the control of a 3-axis micro-manipulator as shown in Figure 8. Force signals acquired from probing these cells were collected and analyzed. Manipulation velocity of the probe may cause different impact force as well as inertia force on the sensing system. The relationship between the sensor output signal and the probing velocity is discussed as follows.

Cell Probing Signals

Unfertilized egg cells of *Danio rerio* (with diameter ranging from 500μm to 1mm) were used in our cell probing experiments. The cells were placed on an agarose dish and observed under a microscope during the probing process. The probing speed of the micro-manipulator was set to 1000μm/s and the travel distance was set to 500μm. Under the microscope, the tip of the probe was first manually aligned to point towards the center of the cell and initially 100μm away from the cell membrane. Then, the probe was commanded to move into to the cell by an interface computer program. The key steps during the cell probing experiments are illustrated in Figure 9.

Signals during the various stages of the probing process were recorded and are shown in Figure 10. A control experiment was performed by moving the sensing system with an attached probe, but not allowing the probe to touch the cell membrane. The result of this experiment is given in Figure 10a. Two large-amplitude vibration signals were found due to the change in inertia force when the probe started and stopped to move. When a Turner Probe touched the cell membrane (Figure 10b), the signal

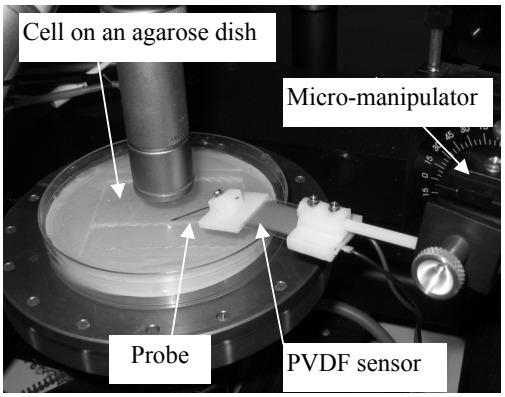

Figure 8. Experimental setup for the cell-probing experiments.

increased suddenly, which corresponded to the impact force on the membrane. Then, the signal dropped because the probe penetrated the cell membrane (Figure 9c) so that mechanical resistance on the probe was lowered on the probe from the membrane. These experiments clearly indicate that the PVDF sensing system is sensitivity enough to pickup the cell-probing signals. With the same configuration as the probing experiment, the probe was drawn out of the cell (Figure 9d) and the obtained signal is shown in Figure 10c. When the probe started to move, the signal dropped because the frictional force between the cell and the probe moved the PVDF sensing element in the reverse direction of the probe withdrawing motion. After the probe moved to a certain distance, it left the cell membrane, so as the force exerted on the probe decreased, a consequent rise in the signal was observed. Similar experiments as the above were carried out using KL Probes. Since KL Probes are much "sharper" than Turner Probes, i.e., they have much smaller tip angle and much longer taper length than Turner Probes, so by intuition, KL Probes should penetrate cells with less mechanical resistance. The signal obtained from our experiments validated this conjecture. As shown in Figure 10d and 10e, both the penetration and extraction signals are much lower than when Turner Probes were used. Hence, KL Probes will cause less damage to a cell during a cell probing/injection process.

As aforementioned, when the probe was commanded to move, the sensing system itself has an inertia force as shown in Figure 10a, whose magnitude depends on the probing speed (the faster the probing speed, the greater the acceleration and deceleration). This inertia force can sometimes give misleading force response when its magnitude is comparable to the cell-probing force. Our ongoing work is to carry out experiments similar to the process described above with various speeds. Initial results indicate that both impact force signal and inertia force signal increased as the velocity increased. However, the impact force signal on the cell increased more than inertia force signal as velocity increased, which is an indication that the cell membranes offer a finite mechanical resistance during the cell probing process even for nanometric scale probes such as KL probes. More detailed quantitative analysis will be performed and our results will be published shortly.

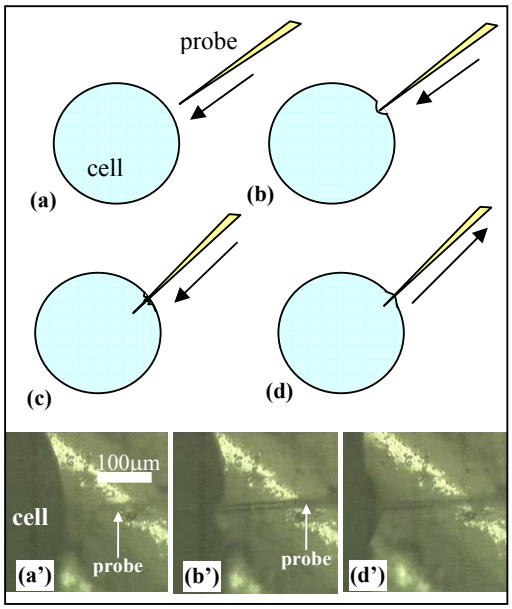

Figure 9. Illustrations showing various stages during a cell injection/probing/dissection process: (a) the probe moves towards the cell (corresponding microscope image is shown in (a')); (b) the probe touches and deforms the cell membrane (corresponding microscope image is shown in (b')); (c) the probe penetrates the cell membrane; (d) the probe is extracted from the cell (corresponding microscope image is shown in (d')).

CONCLUSION

A newly invented chemical probe-etching process with high reproducibility was developed which provides a simple, single-step method to fabricate probes with nanometric tips. With a suitable selection of organic solvent and correct etchant boundary conditions, it is likely to be a promising and quick process to fabricate probes for SNOM applications, cell probing and sensing tools, or even cell surgery purposes. In addition, initial experimental analyses were performed on the impact signal between cell membranes and probes made by Turner's and Kwong-Li's methods. The sensed signals clearly indicate that cell membranes will offer finite mechanical resistance even for the nanometric KL Probes during the probing process. However, KL Probes clearly cause less mechanical

contact force on the cell membranes than Turner Probes.

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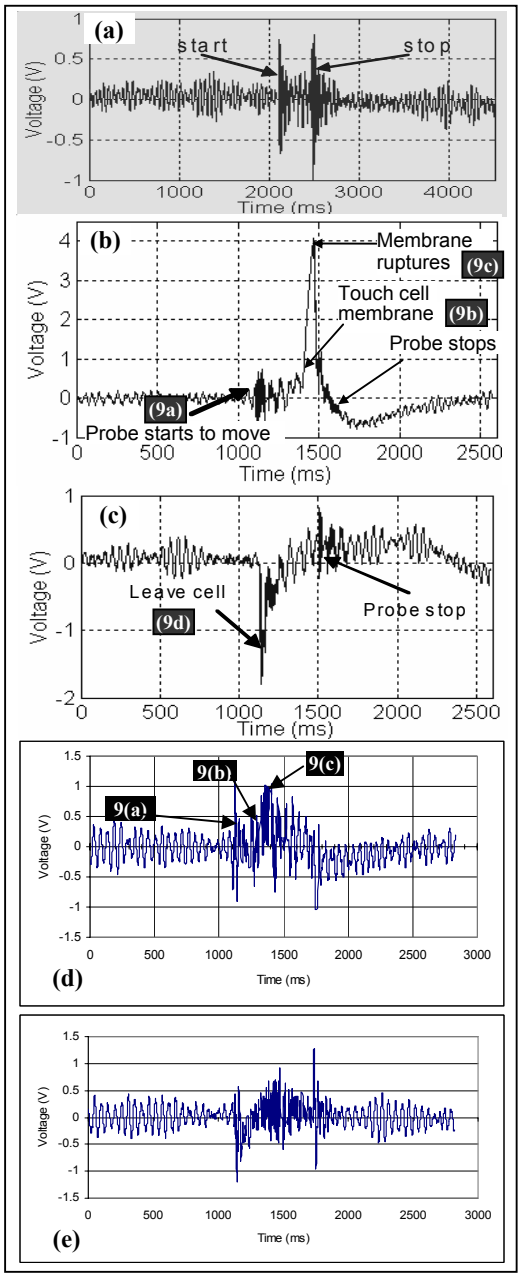

Figure 10. Voltage signal from the PVDF micro-sensing system which was used to measure the force of the probes as they impinge on the membrane of a cell. (a) The inertia force of the manipulation system before the probe touched a cell. (b) A Turner Probe penetrated a cell membrane. The regions on the signal curve can be related to the steps illustrated in Figure 9a, 9b, and 9c. (c) A Turner Probe was extracted from the cell. (d) A KL Probe penetrated a cell membrane. Much lower force signal was recorded, indicating much less resistance from the membrane on the probe. (e) A KL probe was extracted from a cell. Again, much lower signal was observed than using a Turner Probe.