KL Probes for Robotic-Based Cellular Nano Surgery

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***Abstract*—We recently developed a very fast and reliable single-step etching process to fabricate sharp-angled micropipette and probe tips from capillary tubes and optical fibers, respectively. This novel process is less complex and yields finer tips than other available multi-steps sharp-tip (e.g., SNOM tips) fabrication processes. The process is named as Kwong-Li's (KL) Method. 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 capillary tubes into micropipettes with tip angle <2.1° and with tip diameter of 5μm. Optical fibers (with initial fiber diameter of 125μm) were also sharpen into tiny-tips with angles ranging from <2.7 – 9.7°, and with nanoscale tip diameters of <1μm. With their nanometric tips, the KL micropipettes and probes could potentially be used as surgical tools for micro cellular surgery and possibly for scanning probe microscopy applications. We have already shown that KL probes could penetrate through cell membranes with less mechanical resistance than conventional pipettes and probes made from Turner's Method. The fabrication process of KL Method and experimental results from using KL probes to probe cells are presented in this paper.**

Keyword: Sacrificial Boundary Etching; SNOM Tips; Nano Tips; Kwong-Li Probes; Kwong-Li Method; Micropipette

I. INTRODUCTION

The fabrication of micro/nano scale probes/micropipettes has become essential in intracellular surgery. The size of micro probes is a major limitation for micro-cellular operation. For example, to investigate the DNA or small biological tissues in medical sciences, a probe with ultra-small tip diameter is needed. The micropipette has been used in the past but the applications were limited by its size. A summary of micropipette applications in neural sciences is given in [1]. Currently, the micropipette is being used in two major ways: 1) it acts as a microelectrode to obtain electrical signal; 2) to perform fluid/substance injection that allows fluid/substance to transfer from one end to the other end which contact with the cell. Thus, improved technologies for cell sensing, manipulation, or injection could be realized by minimizing the size of these probes and micropipettes.

On the other hand, Scanning Near-field Optical Microscopy (SNOM) was invented in 1984 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 achievable spatial resolution. And, 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 is combined with 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 probes and micropipette etched by KL Method, such as tip profile, tip diameter and tip angle, are discussed in this paper.

II. PRINCIPLE OF KL METHOD

A simple chemical etching process was introduced by Turner using pure Hydrofluoric (HF) acid and organic solvent to fabricate small probe tips from optical fibers [5]. Interfacial meniscus formed between an organic solvent layer and HF which was employed to etch the fibers. The stripped fiber was dipped into the etchant and the final probe tip was 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. In fact, P. K. Wong et al., have demonstrated etching of micropipettes using this principle in [6].

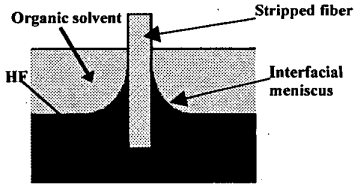

Figure 1. Illustration of Turner's Method.

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

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probe profile, we can simply adjust initial HF height (h) in the glass tubing as defined in Figure 2.

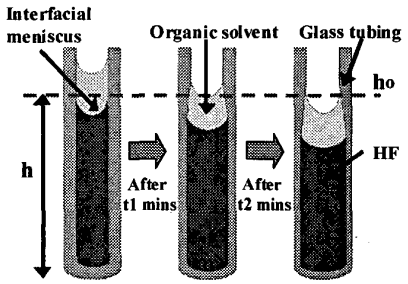

Figure 2. Controlling the interfacial meniscus by sacrificial boundary etching.

III. EXPERIMENTAL RESULTS

A set of sacrificial boundary etching experiments was conducted with glass tubings of 21mm inner diameter. Fiber optics and fused silica capillary tubings were the samples used to test the KL method. Fiber probes were also fabricated by Turner's Method as reference probes to KL probes. In all experiments, single mode fiber was used (F-SA with cladding diameter of 125μm, Newport Corporation) to fabricate KL probes; flexible fused silica capillary tubing was used (TSP002150 with inner diameter of 2μm and outer diameter of 126μm, Polymicro Technologies) to fabricate KL micropipette. The difference between probe and micropipette is that the micropipette contains a hollow channel which allows fluid to pass through it. 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 front views of a KL probe, a Turner probe, and a pulled glass pipette is shown in Figure 4. 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. KL method was also applied to fabricate micropipette as shown in Figure 5 and Figure 6. KL micropipette will also allow biologists to conduct experiments with much smaller cells than Turner probes.

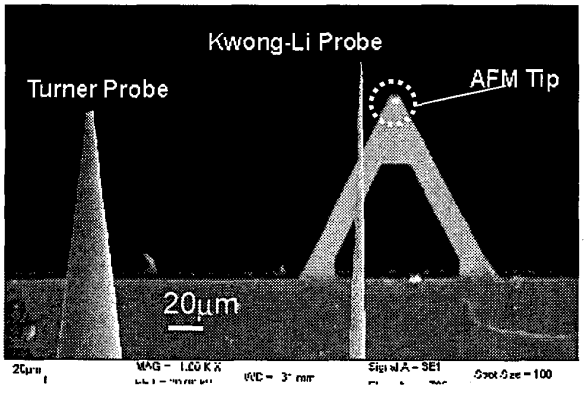

Figure 3. SEM picture of KL probes and Turner's probe.

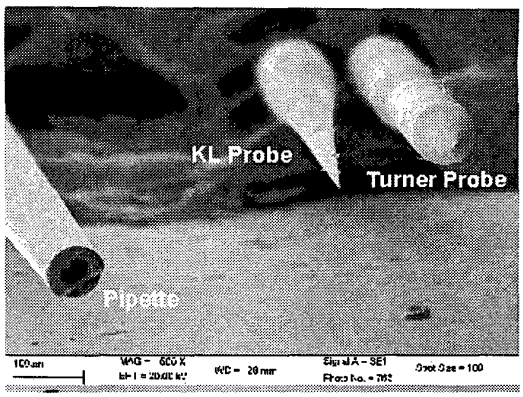

Figure 4. SEM picture of a KL Probe, a Turner Probe, and a conventional pipette. The scale bar is 100μm.

A. *Tip profile*

The probes fabricated by KL method have long tapers and very sharp angles and are described in [7]. Taper of fabricated KL probe can be longer than 2mm, which is much longer than a typical Turner probe, whose length is just 332.5μm as limited by Turner's process. The tip diameter is another difference in the profiles of KL probes and Turner's probes. By using the p-xylene and HF for the chemical etching, typical tip diameter of Turner probe is about 1μm. However, tip diameters ranging from 500nm to 1.5μm can be obtained for KL probes. Taper of the fabricated KL micropipette is about 1.5mm as shown in Figure 5. The cavity at the tip end is also enlarged due to the chemicals diffusing into the cavity during the KL process. For KL pipette as shown in Figure 6, the inner tip diameter is about 5.5μm and outer tip diameter is about 6.5μm.

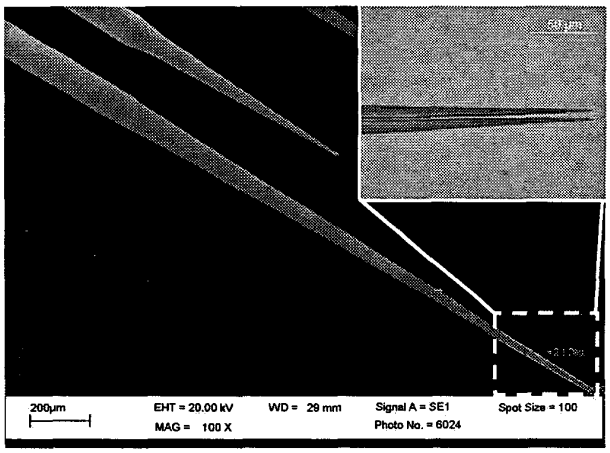

Figure 5. SEM picture of a KL micropipette showing the tip angle. The inset is an inverted microscope picture showing the inner microchannel profile of the KL micropipette.

B. *Tip angle*

Another great advantage of the KL Method is that the final probe 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). Similarly, the fabricated KL micropipette tip angle can be sharpened down to <2.1°. 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 micropipette tip angle versus h is given in Figure 7.

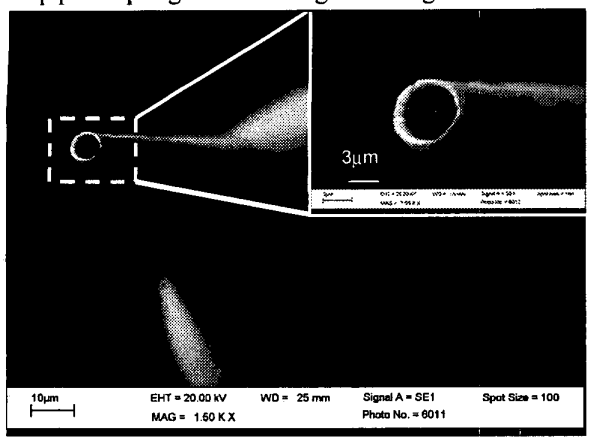

Figure 6. SEM picture of a KL micropipette showing the tip hole.

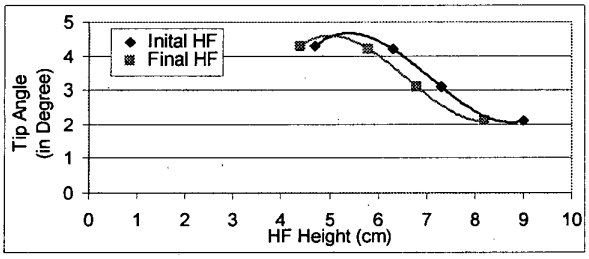

Figure 7. Relationship between KL pipette tip angles and HF height.

IV. Cell-Probing Experiments

Excessive force is one of the factors that kill the cells during the probe penetration of biological tissues. A micromanipulation station with a μN sensing system was developed in our prior work [8] 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).

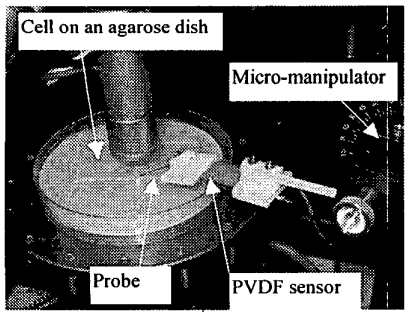

Figure 8. Experimental setup for the cell probing experiments.

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.

A. 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 micromanipulator 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 the cell by a computer program. The key steps during the cell probing experiments are illustrated in Figure 9.

Signals in 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 to move and stopped from moving. When a Turner Probe touched the cell membrane (Figure 10b), the signal 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 sensitive enough to pick up 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 that 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, and the force exerted on the probe decreased, then a consequent rise in the signal was observed. Similar experiments 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.

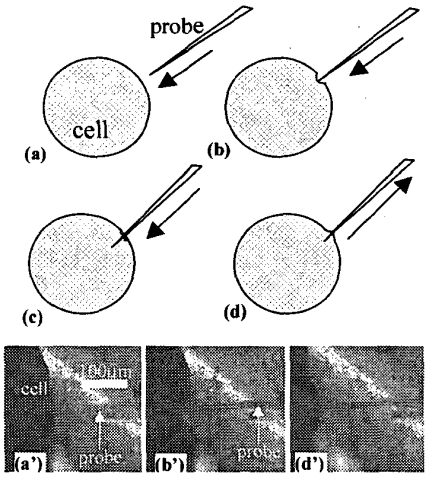

Figure 9. Illustrations showing various stages during a cell injection/probing/dissection process: (a) the probe moves towards the cell (corresponding microscope image is shown in (a')); (b) the probe touches and deforms the cell membrane (corresponding microscope image is shown in (b')); (c) the probe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

V. 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.

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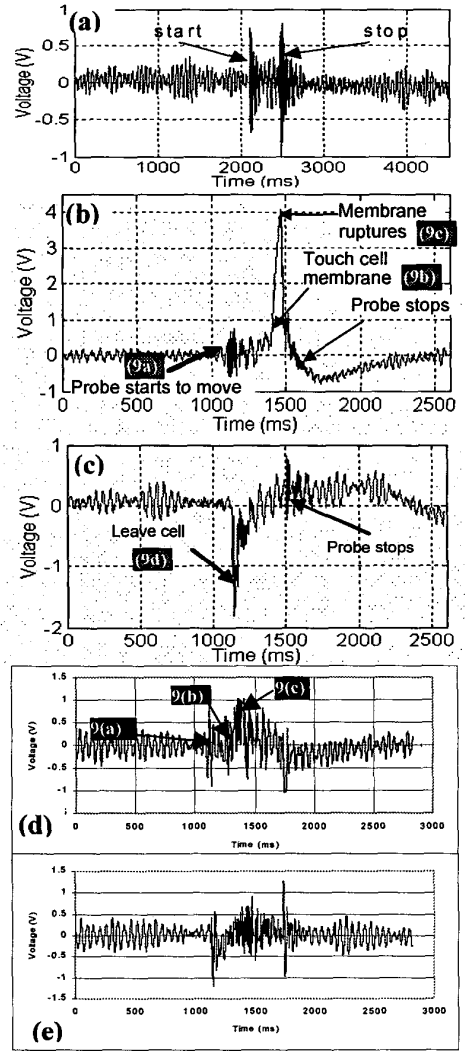

Figure 10. Voltage signal from the PVDF micro-sensing system which was used to measure the force of the probes as they impinge on the membrane of a cell. (a) The inertia force of the manipulation system before the probe touched a cell. (b) A Turner Probe penetrated a cell membrane. The regions on the 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.