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Membrane Oscillator as a Chemical Sensor

Part 1: Fabrication of TM Pipet

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ABSTRACT

A rhythmic, sustained, stable oscillation was reproducibly observed for a lipid membrane supported by a micropore of a thin membrane tip micropipet (TM pipet). The construction of the TM pipet was accomplished by implementing a microfabrication method that allowed the transference of a Si_3N_4 film with a hole from the Si substrate to the glass tube tip. The main part of the fabrication method is the sealing process: a mix between thermal and amodic bonding. The TM pipet fabrication is described in detail with emphasis on the thermal-anodic bonding process. In addition, a general account of the new device's main features, including various applications, is given.

INTRODUCTION

Since biological systems are very complicated, it has been found profitable to construct artificial systems mimicking biological organs in order to clarify their mechanisms. The typical examples are the studies of the receptor and neural excitation mechanisms. Useful devices, such as sensors and information processing devices, are expected to appear from the studies.

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Artificial membranes have been used in the studies of biological excitation or oscillation mechanisms (*1–4*), but reproducibility has been poor. Yoshikawa (*5*) and Kawakubo (*6*) reported spontaneous oscillation of electrical potential across a liquid membrane using a U-shaped glass tube. The reproducibility of their system was considerably improved in contrast with previous studies, but the oscillation continued only for several hours. Shimoide and Moriizumi used a single micropore in a Si_3N_4 membrane in order to support the lipid material (*7*), and found that the reproducibility of oscillation was exceedingly improved in comparison to a multipore system, such as the nucleopore membrane (*4*), although the oscillation was not as stable as the liquid-state membrane.

We have reported (*8,9*) that a rhythmic, sustained, stable potential oscillation was observed for a lipid membrane supported by a thin membrane tip micropipet (TM pipet), and found that the stable electrical oscillation could be maintained for more than 120 h using the pipet.

The present report consists of two parts. First we review the fabrication method and the fundamental properties of TM pipet in Part 1, and second, the details of observed oscillation phenomena are discussed in Part 2.

BASIC SCHEME OF THE TM PIPET

Since the discovery of the field assisted or anodic bonding (*10*), the transference of thin solid films grown on metal or semiconductor to glass substrates was an underlying possibility. However, imperfections on the contact surfaces, trapped dusty particles, and so forth, produces unpredictable void regions, reducing the applicability of the technique. Recently, the authors reported a new technique that solved the problem (*11*). In short, the novel technique consists of improving the contact between the parts by applying a heat pulse (melting only the glass at the interface), and then strengthening the seal using anodic bonding.

Using this technique, a new type of micropipet (TM pipet) could be implemented. It consists of the transference of a thin silicon nitride membrane, where a hole was already drilled by electron beam (EB) lithography and reactive ion etching (RIE) processes, from the silicon substrate to the tip of a glass tube, yielding a TM pipet.

Fig. 1 shows the TM pipet and, for comparison, the conventional glass micropipet. The conventional glass micropipet have already been established as sensing devices in the chemical and biology fields. For instance, in biology, they can be used to study the mechanical properties of the cell membrane or to record the cell membrane ion channel activity. However, since they have a long "shank" region, their electrical resistance is usually high, and at the tip, their electrical parameters are distributed in form (*see* the magnified insert in Fig. 1). On the other hand, the TM pipet, which

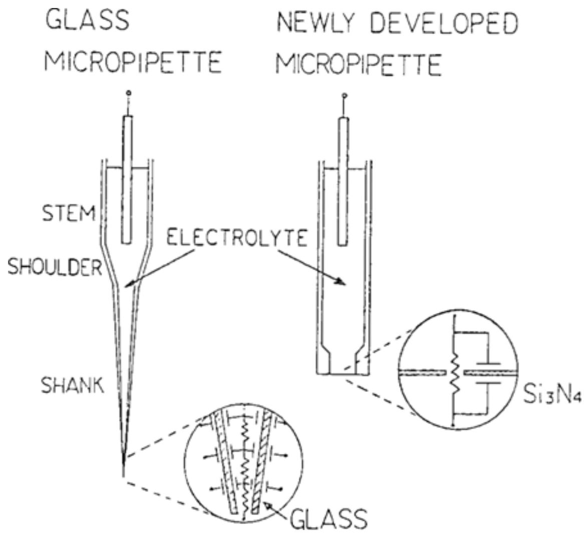

Fig. 1. Conventional and TM pipets. The magnified inserts show the equivalent circuits of both devices at the tip.

does not have shank region, has a comparatively lower resistance and a much simpler electrical model (without distributed parameters). Measurements of the TM pipet electrical characteristics have shown that it has a resistance approx 10× lower than that of a conventional glass micropipet, that the capacitance is not dependent on the tip immersion depth, and that the tip potential is closer to a salt bridge junction potential (*12*).

TM PIPET FABRICATION

The TM pipet fabrication process is schematically sketched in Fig. 2.

First, a silicon nitride membrane (usually 150 nm thick) is grown on a silicon substrate by low pressure chemical vapor deposition (LPCVD). Then a ring and hole located at its center are written on this membrane by EB and RIE. On the other branch of the fabrication procedures (*see* Fig. 2), a glass tube tip (10 μL Microcaps, Drummond Sci. Co., id 560 μm) is submitted to heat treatment, in order to facilitate filling the micropipet with solution by adjustment of the contact angle between liquid and the tube internal wall. The glass tube tip is held close to a heater where it softens, reducing in diameter. The final tip diameter is a function of the heating temperature, time, and the separation distance. The results are plotted in Fig. 3(A). Fig. 3(B) shows the final glass tube photomicrograph. Glass tube tips heat-treated to diameters varying from 100 to 200 μm were used during the experiments.

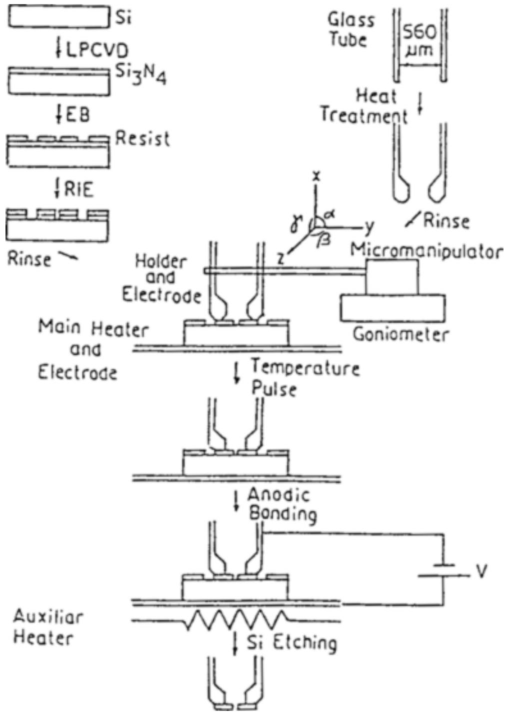

Fig. 2. TM pipet fabrication process.

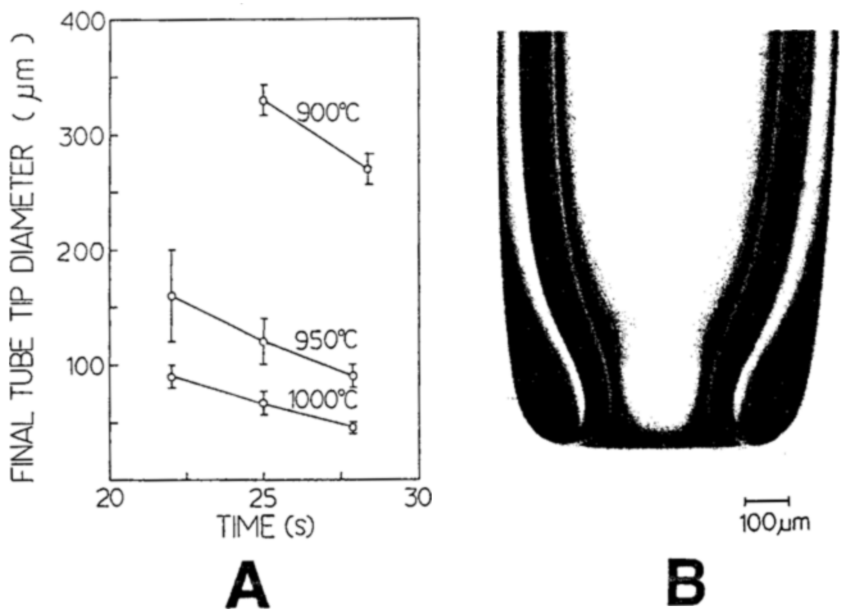

Fig. 3. (A) Relation between the TM pipet tip diameter and heat treatment duration for a 200 ± 50-μm separation distance. (B) Glass tube tip photomicrograph.

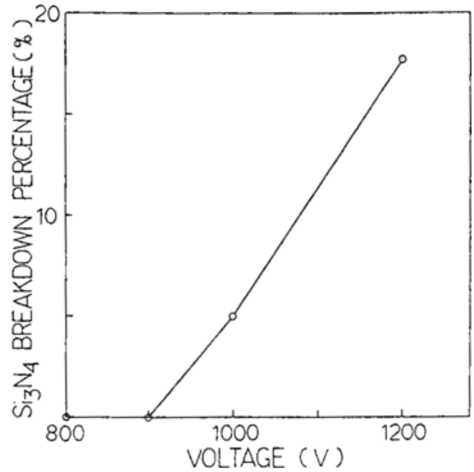

Fig. 4. Si_3N_4 breakdown percentage as a function of the applied voltage.

Subsequently, the glass tip is brought in contact with the silicon nitride film by means of a micromanipulator settled on the top of a goniometer (*see* Fig. 2). The precise location is monitored by a microscope, using the ring as a visual guide. Applying a pulse of temperature (850°C for 1 s) yields a good and larger contact area between the parts.

The next step is to perform the anodic bonding. The temperature at the contact interface is chosen to be close to the annealing point of the glass (521°C for Pyrex 7740 glass), where all the atoms inside the glass are believed to be ionized. The bonding voltage is limited by the breakdown of the nitride film. Because of the unpredictability of the electrodes' contact resistance, this limit can vary from one experiment to another. The percentage of breakdowns as a function of the external applied voltage, under the present experimental conditions, is plotted in Fig. 4.

Taking the above results into account, the anodic bonding was performed at 520±5°C and 800 V. The bonding quality was inspected by etching all the silicon substrate back. Strong bonding results in the silicon nitride membrane left over the glass tube tip, forming the micropipet. The percentage of bonding success was found to be dependent on the bonding time. Combining those results, it can be said that the delivered amount of charge during the bonding is the factor controlling the bonding quality. The charge amount needed to yield a strong seal was estimated to be more than 200 μC from current measurement during the bonding.

Finally, Fig. 5 shows the Si_2N_4 film transferred to the glass tube tip, yielding the TM pipet.

ELECTRICAL CHARACTERISTICS OF THE TM PIPET

The electrical characteristics of the micropipet were measured using the experimental arrangement shown in Fig. 6.

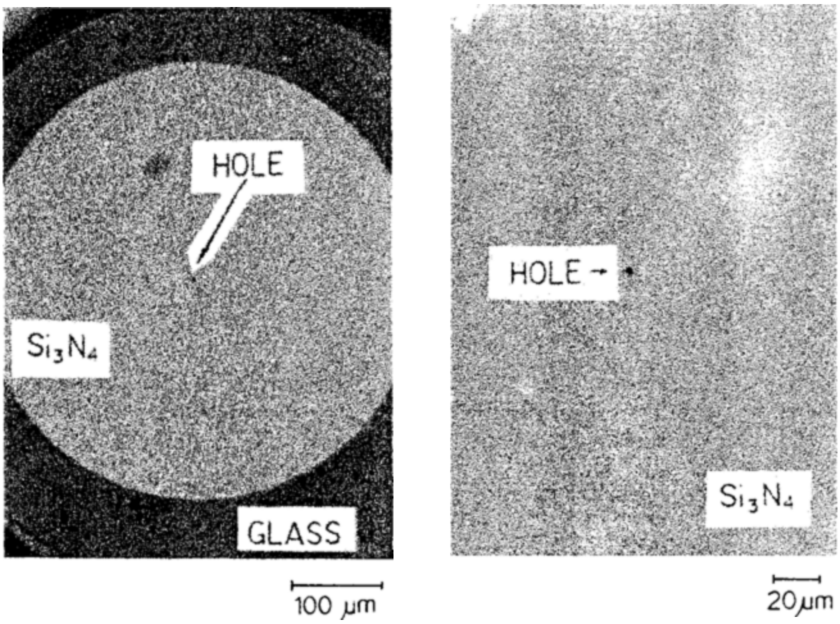

Fig. 5. Final TM pipet top view.

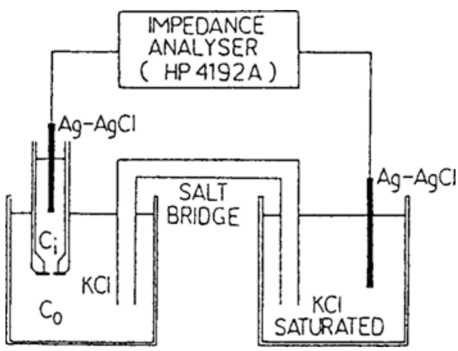

Fig. 6. Arrangement to measure the electrical characteristics.

Figure 7 (a) shows the resistance as a function of the pore diameter, and the lower and upper limit curves were drawn following the theoretical prediction (*13*). The capacitance was dependent on the glass tube tip diameter as shown in Fig. 7 (b).

Comparing the results shown in Figs. 7 (a) and (b) with the electrical characteristics of the conventional glass micropipet described in the literature (*14*) shows that the present micropipet has a resistance at least one order of magnitude lower and that its capacitance depends on the capacitance of the Si_2N_4 membrane at the tip.

The impedance as a function of the frequency is plotted in Fig. 8 (a) where the lines represent the calculated values for a pipet with a 1-μm

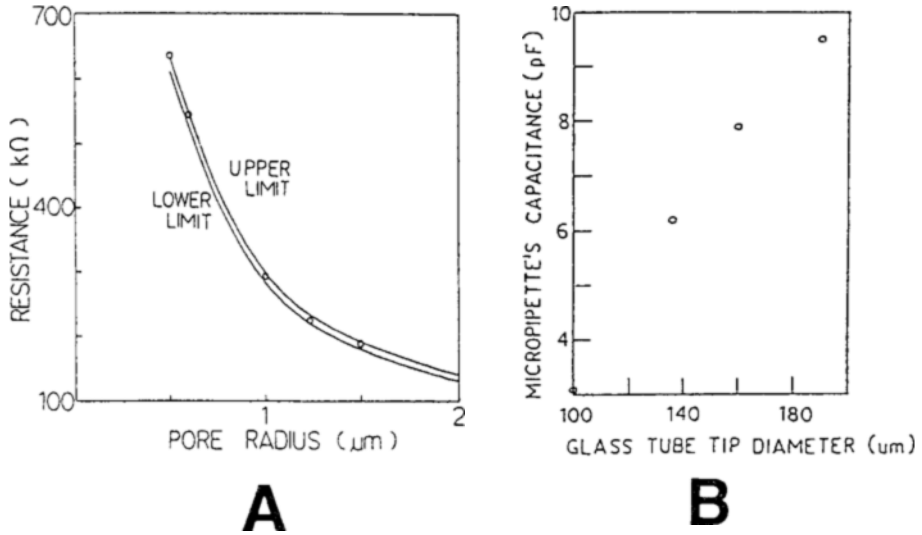

Fig. 7. (a) Resistance vs pore radius and (b) capacitance vs diameter of the glass tube tip for the TM pipet.

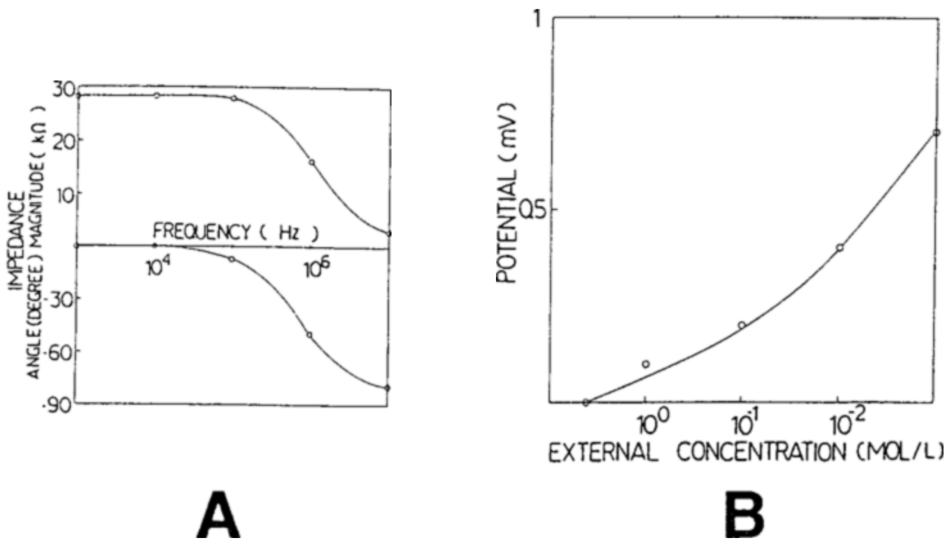

Fig. 8. (a) Frequency characteristics of the TM pipet impedance, and (b) tip potential as a function of electrolyte salt concentration.

diameter pore filled with 4.2*M* KCl solution. This results shows that it can be used on measurements up to 350 kHz, whereas a corresponding glass micropipet cannot reach such a value.

The experimental setup used to measure the TM pipet tip potential was essentially the same as that shown in Fig. 6, but the reference solution concentration was changed to 4.2*M* KCl. The salt bridge was also made of

4.2*M* KCl and the measuring instrument was a high input impedance ($10^{12}\Omega$) differential amplifier connected to a voltmeter. Using this arrangement the difference between the potential of the salt-bridge/test-solution junction potential and the pipet tip potential was measured. The results are presented in Fig. 8 (b), and show an increasing potential difference with the decrease in test-solution concentration. This potential remains, however, at a low level (<1 mV). For a glass micropipet such potential difference is expected to be on the order of tens of millivolts.

DISCUSSION AND CONCLUSION

The features of the TM pipet can be understood when compared with those of conventional glass micropipets (*see* Fig. 1), and were confirmed experimentally in the present study. Since the TM pipet does not have a shank region, its electrical characteristics are better than those of the conventional micropipet. The TM pipet has resistance at least one order smaller than a glass micropipet, and the cutoff frequency of a TM pipet is about one order higher, since the capacitance values are almost comparable. In addition, TM-pipet tip potential is in good agreement with the theoretically predicted values and much smaller than that of a glass micropipet. Hence, the TM pipet can be utilized not only for a microreference electrode but for a precise potential measurement; for example, extracellular potential mapping.

The TM pipets were applied as a potassium ion-selective electrode (ISE) (*12*), and both good K^+ ion selectivity and a response time faster than a glass micropipet were obtained. In the investigation of red blood cell (RBC) mechanical properties (*15*), the TM pipet can be considered superior to that expected when a conventional micropipet is used. For instance, a RBC could pass through the 1-μm diameter pore of the TM pipet, but it cannot go through a glass micropipet, because the latter shank restricts the cell flow. A preliminary study showed that the TM pipet can be used for RBC measurements (*16*).

The promising applications of the TM pipet, both verified and expected, are listed in Table 1. The study described in the next chapter is related to the application placed at the bottom of the table.

ACKNOWLEDGMENTS

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Table 1
TM Pipet Promising Applications

Function	Application	Comments
Microelectrode	Measurement of localized potentials	
	Reference electrode	
	Ion selective electrode	Suitable ion selective membrane placed at the TM pipet tip
Micromechanical tool	Measurement of cell membrane mechanical properties	
	Localized pressure sensor	The in-out pressure difference changes the ionic diffusion rate changing the TM pipet resistance.
	Cell handling	Holding cells in a determined position for electrical and/or optical tests
Others	Microsampling or microinjection	Sampling of small volumes, or pressure or ionophoretic injection of chemicals
	Microreaction chamber	Holding small volumes of chemical substances at the TM pipet tip which can react with the external solution through its pore

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