

## Fabrication of a Shear Force-based Ion-selective Capillary Probe for Scanning Electrochemical Microscopy

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A shear force-based ion-selective probe for scanning electrochemical microscopy was fabricated for the imaging of localized potassium ions. A solution of dibenzo-18-crown-6/1,2-dichloroethane was added to a pulled capillary which was contact with a tuning fork attached to a piezoelectric buzzer. 10  $\mu\text{m}$  of a Nafion island on glass was imaged, and the topography and current image originating from localized potassium ion concentration were obtained.

Scanning electrochemical microscopy (SECM) has been employed in the investigation of biological systems. Solid microelectrodes such as platinum or carbon have been used to probe redox reactions catalyzed by enzymes<sup>1–6</sup> and electroactive species such as oxygen.<sup>7–9</sup> Probing a non-electroactive ion such as potassium, sodium, or calcium is important since these species are employed in various physiological activities. A micropipette filled with a solution of ionophore dissolved in organic solvent has been used to probe non-electroactive ions in SECM.<sup>10–12</sup> There have been reports of probing ion transfer through a bilayer lipid membrane,<sup>10</sup> a small pore,<sup>11</sup> and a biological tissue.<sup>12</sup> To image the concentration change of these ions around cells, distance control between the tip of the probe and the sample is crucial. Shear force control is commonly used to regulate the distance between a tip and a substrate.<sup>13–18</sup> In this report, a shear force-based ion-selective micro/submicro electrode is fabricated, and an island of Nafion on glass is imaged using a standing approach mode<sup>7,13,14</sup> as a model of living cells.

All the aqueous solutions were prepared with water purified using a Milli-Q Jr (Millipore Co.). Magnesium chloride hexahydrate, dibenzo-18-crown-6, 1,2-dichloroethane, and 20% Nafion dispersion solution (DE2021CS) were purchased from Wako Chemical, Tokyo, and were used as received. Tetraoctylammonium tetrakis(4-chlorophenyl)borate was prepared as follows: Saturated solutions of tetraoctylammonium bromide/ethanol and tetrakis(4-chlorophenyl)borate potassium salt/ethanol (Dojindo, Tokyo) were mixed, and the precipitate was recrystallized three times from ethanol.

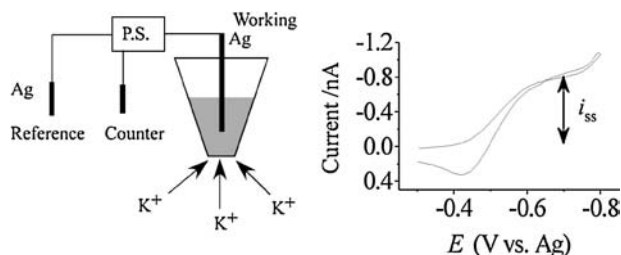
A glass tube (1.5-mm diameter, Terumo, Japan) was pulled with a capillary puller (Narishige, Tokyo model PC-10) so as to form a 0.2–2- $\mu\text{m}$  capillary tip. The pulled capillary was filled with a mixture of 25–50 mM dibenzo-18-crown-6 (DBC) and 25 mM tetraoctylamine tetrakis(chlorophenyl)borate (TOA-TCPB) in 1,2-dichloroethane (DCE), and 0.1-mm Pt wire was inserted into the capillary for electrical contact. A quartz crystal tuning fork (Citizen, CFS-308) was soldered onto a diaphragm of commercially available piezoelectric transducer in order to excite the mechanical resonance of the tuning fork, and the pulled capillary was contacted with one prong of the tuning fork (Figure S1 in the Supporting Information). Since the capillary

is not glued to the tuning fork, replacement of the capillary is easy. A Nafion island on glass was prepared as follows: A Nafion solution was introduced to a thick glass tube (10- $\mu\text{m}$  i.d., 100- $\mu\text{m}$  o.d.) by dipping the tip of the glass tube in a 20% Nafion solution. After drying for 2 h, the tip of the thick glass tube was polished on a lapping film (Sumitomo 3M, Tokyo), and it was set at the bottom of the electrochemical cell.

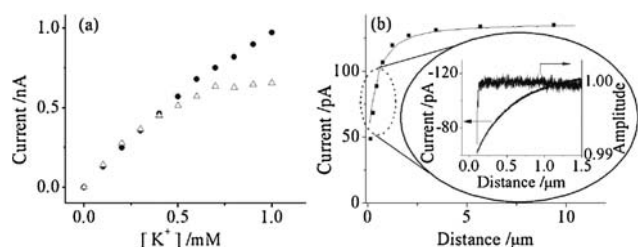
An SECM system (Hokuto Denko, HV401) was modified in order to use shear force-based tip positioning. A piezo actuator (Piezomechanik GmbH, model PSt 150/7/20 VS12) was mounted on a stepping motor-driven XYZ stage (Suruga seiki, KS701-20LHD) to perform the micromovement of the probe along the Z axis. The stroke of the piezoelectric actuator was 7  $\mu\text{m}$  at an applied voltage of 150 V. The signal of the tuning fork was amplified with a lock-in amplifier (Signal Recovery, Model 7264), and then, the signal amplitude was digitized and transferred to a computer (Toshiba Dynabook) via a 16-bit AD/DA board (Interface, AZI3506). The measurement was carried out in a three-electrode configuration. The quasi reference and counter electrodes were composed of Ag and Pt wires (0.5-mm diameter), respectively. The tuning fork and the tip of the electrode were vibrated at the resonance frequency (20–30 kHz), parallel to the sample surface. The applied voltage of piezoelectric transducer (0.02–0.1 V) was adjusted to be the starting amplitude of the tuning fork at 0.95 mV.

The standing approach mode described previously was used to image the electrochemical activity and topography of the surface.<sup>13,14</sup> This mode repeats at each point the tip approaches and retracts to sample the current. Lateral movement of the tip is stopped during the data-sampling sequence. The starting amplitude of the tuning fork is measured before the approach is initiated, and then the tip is lowered to the surface at a rate of 0.5  $\mu\text{m}/\text{s}$  until the amplitude of the tuning fork is damped to 99% of the starting amplitude. The tip is then retracted by 0.2–0.3  $\mu\text{m}$  to acquire the current. When the tip is moved laterally to the next sampling point with the stepping motor, it vibrates at the beginning and end of the movement as a consequence of the vibration of the stepping motor. Therefore, the tip is retracted ca. 2  $\mu\text{m}$  upward to avoid contact between the tip and the surface during the lateral movement. It takes ca. 1 s to get a data point without contacting the surface. The electrochemical measurement was carried out in 50 mM aqueous  $\text{MgCl}_2$  solution as a supporting electrolyte.

When the pulled capillary, filled with DBC and TOA-TCPB solution, was immersed in an aqueous solution of potassium ion and the potential of the Ag electrode in the capillary was scanned from  $-0.3$  to  $-0.8$  V vs. Ag, a steady-state current originating from the diffusion of potassium ion in the aqueous solution was observed (Figure 1). The current at the capillary depends on the concentration of potassium ion in aqueous solution and



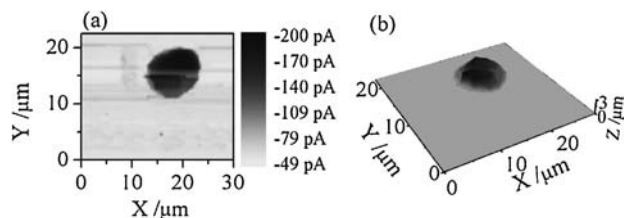
**Figure 1.** Schematic representation of the electrochemical measurement (left) and a cyclic voltammogram of the pulled capillary, filled with 50 mM DCE and 25 mM TOA-TCPB in DEC solution, immersed in 0.5 mM KCl/50 mM MgCl<sub>2</sub> aqueous solution (right).



**Figure 2.** Dependence of the steady-state current on the concentration of potassium ion when the concentration of DBC was 25 mM (open triangles) and 50 mM (solid circles) (a) and the current–distance profile when the tip of the capillary was brought near a glass surface at  $-0.7$  V vs. Ag (b).

the concentration of DBC in DCE. When excess DBC is present in DCE, the current depends only on the concentration of potassium ion in aqueous solution and a steady-state current (*i*<sub>ss</sub>) is obtained owing to spherical diffusion around the micrometer-scale tip of the capillary. To conform that the *i*<sub>ss</sub> is the diffusion-limited current of potassium ion in aqueous solution, the dependence of the steady-state current of the capillary probe on the concentration of potassium ion was measured. As shown in Figure 2a, linearity was observed up to 0.8 mM when the concentration of DBC was 50 mM. On the other hand, when the concentration of the DBC was 25 mM, the current was independent of the concentration of potassium greater than 0.6 mM. This phenomenon indicates that DBC at the interface is consumed when the concentration of potassium ion is higher than 0.6 mM. Figure 2b shows the current–distance profile when the tip approaches the glass until the amplitude of the tuning fork is damped to 99% of the starting amplitude. The solid square in Figure 2b represents the theoretical value when the inner and outer radii of the capillary are 0.63 and 1.3-μm, respectively. The change of the current and the amplitude of the tuning fork when the distance is very near the glass surface are also shown in Figure 2b (inset) and Figure S2 in Supporting Information. Next, the current–distance curve was measured when the tip was brought near a Nafion membrane on glass. In this case, the current did not decrease with decreasing distance between the tip and the membrane due to the flux of potassium ions from the Nafion membrane.

Figure 3 shows current and topographic images of the Nafion island on glass obtained with a capillary having inner and outer radii of 0.7 and 1.4-μm, respectively. Since the Nafion



**Figure 3.** A current image (a) and a topographic image (b) of an island of Nafion on glass in 0.5 mM KCl/50 mM MgCl<sub>2</sub> solution obtained with a 0.7-μm i.d./1.4-μm o.d. capillary.

membrane swelled in aqueous solution, a small protrusion ca. 2 μm high was observed. At the Nafion zone, the current was large compared to glass because of the flux of potassium ions from the Nafion membrane.

A small Nafion island, as a model of a living cell, was imaged with a shear force-based ion-selective electrode using standing approach mode, and current and topographic images were obtained simultaneously. This system could be useful for imaging the concentration of non-electroactive ions around living cells.

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- 19 Supporting Information is available electronically on the CSJ-Journal Web site, <http://www.csj.jp/journals/chem-lett/index.html>.