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Investigation of ion current behavior on charged samples using scanning ion conductance microscopy

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Abstract– The scanning ion conductance microscope (SICM), a member of the family of scanning probe microscopes, can be used to study nano and micrometer scale biological samples. SICM images samples by detecting ion current flowing through a pipette aperture. However, the electrical charge of the sample surface influences the ion current detected by SICM.

In this research, to investigate the influence of the charged sample on SICM imaging, the behaviors of the ion current on charged surfaces were studied. For the approach curve, current behavior as the nanopipette approaches the surfaces, the detail properties including non-linearity and its influence on imaging were investigated. Imaging of Indian muntjac that is spontaneously charged negatively in buffer solution was performed. When positive potential was applied to the pipette electrode, the topography of chromosome was successfully obtained. On the other hand, when negative potential was applied to the pipette electrode, it was difficult to obtain the topography of the chromosome.

1. Introduction

Scanning probe microscopy (SPM) refers to a family of microscopy techniques that scan a probing tip over a sample surface to provide information about the local characteristics of solid samples. Among various SPM techniques, atomic force microscopy (AFM) [1] has been widely used for biological studies because it can obtain sample topography at nanometer scale resolution [2,3]. In particular, since the AFM works in various environments such as vacuum, air and liquid, biological soft samples have been observed in physiologically relevant aqueous conditions [4,5]. However, it has remained difficult to image the nanoscale topography of larger meso- and microscale biological samples by AFM, because there may be artifacts due to the force between the tip and sample, as well as other factors such as tip geometry and lateral forces [6,7]. To overcome these technical difficulties, some previous investigators have been interested in scanning ion conductance microscopy (SICM). SICM is another type of SPM technique that was first introduced by Hansma et al. [8], and is based on a glass micropipette that serves as a sensitive probe. The signal is modulated by an ion current that flows between an electrode located within the pipette and a bath electrode for feedback control of the pipette-sample distance (Fig. 1). The distance is maintained at the radius of the pipette during scanning, which allows noncontact imaging of the sample topography under liquid conditions. Biological application of SICM was first reported by Korchev et al. [9], who observed live cultured cells under liquid conditions. Since then, SICM has been used to image the surfaces of different cultured cells such as neurons and cardiomyocytes [10-14]. SICM can image samples without damaging by detecting ion current which flows through a pipette with an aperture. However, there is a possibility that electrical charge of the sample surface influences the ion current detected by SICM.

In this research, to investigate the influence of the charged sample on SICM imaging, the behaviors of the ion current on the charged surface were studied. In this report, we discuss the details of the non-linear relationship between the electric charge of the surface and SICM imaging.

2. Experimental procedure

2.1. Scanning ion-conductance microscopy (SICM)

The SICM, which uses a glass nanopipette as the sensitive probe, was suitable for imaging non-conducting surfaces bathed with electrolytes. The SICM utilizes ion-current flow through the pipette aperture. When the pipette edge approaches a sample surface within a distance between the pipette edge and the surface that is less than the aperture diameter, the ion current is reduced. By detecting the drop in the ion current, the operator can control the distance between the pipette edge and the cell membrane with nanometer-scale accuracy. Figure 2 shows the experimental setup of the SICM mounted on a sample stage of an inverted optical microscope (IX-71, Olympus). Biological samples prepared in a petri dish are put on an X-Y flat scanner (NIS-70, Nanonics). The sensitive SICM probe consists of a nanopipette filled with electrolyte with an Ag/AgCl electrode inserted into it.

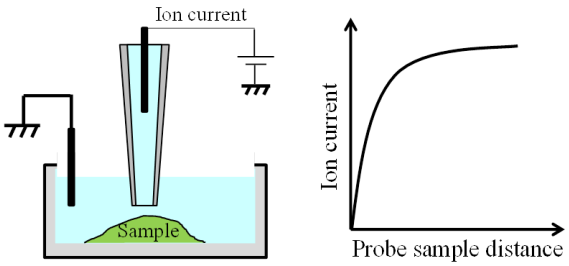

Fig. 1 Schematic representation of SICM

The glass pipette was fabricated from borosilicate capillaries (inner diameter 0.6 mm, outer diameter 1.0 mm, Narishige, Tokyo, Japan) using a CO_2-laser-based micropipette puller (P-2000, Sutter Instruments, Novato, CA, USA). The diameter of the aperture of the pipette edge was about 100 nm. A DC bias electrode is applied between the bath electrode and inner electrodes of the nanopipette. The inner electrode is connected to a high-impedance head stage current amplifier (Axon patch 200B, Axon Instruments, Inc.) that detects the ion current passing from the liquid medium through the pipette aperture. The ion current of the probe close to the sample surface is amplified and then fed into a homemade SICM controller. The controller consists of a comparator, microcomputer, and 16-bit D/A converter. The output signal of the controller is fed to the piezo driver to drive a z-axis piezo actuator (Cedrat Technologies) holding the nanopipette probe. There are several operating modes for SICM. In the direct current (DC) mode, DC ion current is recorded during scanning, while, in the alternating current (AC) mode, the amplitude of the AC ion current modulated by oscillating the pipette probe is detected by using a lock-in amplifier [15]. In the hopping or backstep mode, ion current is recorded while the pipette is moved vertically and repeatedly approaches and retracts from the sample surface [16, 17]. In the present study, images were obtained in the hopping mode. In the hopping mode, the probe approached towards an insulating surface until the ion current reduction is exceeded a predefined threshold. The current reduction of 2% with respect to the basal current was used in this experiment to stop the approach.

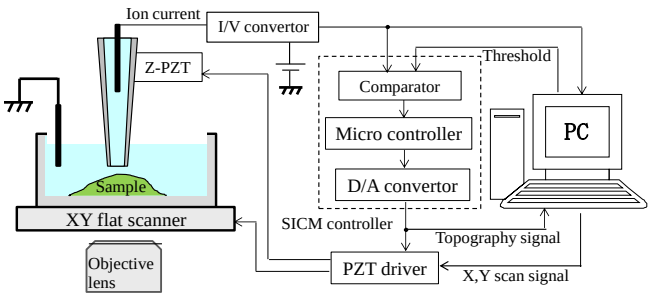

Fig. 2 Experimental set up of a homemade SICM

2.1. Cultured HeLa cell

To examine the image quality of SICM for cultured cells, fixed HeLa cells were imaged by Hopping SICM in this study. At first, HeLa cells were grown on cover slips in culture dishes (50 mm in diameter) for 24‐48 h in a CO_2 incubator at 38◦C, fixed with 1% glutaraldehyde in 0.1 M phosphate buffer (PB) at pH 7.4 for overnight at 4 ◦ C, and observed by SICM.

2.3. Preparation of the Indian muntjac chromosome

Chromosomes of Indian muntjac were prepared according to the standard air-drying method for light microscopy [18]. Briefly, Indian muntjac cells after cultivation were arrested in metaphase by adding colcemid to the culture medium at a final concentration of 0.05 μg/ml for 1 h, exposed to 75 mM KCl for 30 min at room temperature and fixedwith Carnoy's solution (methanol: acetic acid = 3:1, v/v). Chromosome spreads were then formed by dropping the cell suspension onto glass slides, briefly dried in air in order to fix them onto the glass slides. They were immersed in PBS and observed by SICM.

3. Experimental results and discussion

3.1 SICM imaging

Figure 3(a) and (b) show the topographic data of the HeLa cell obtained in PBS with physiological saline concentratin (154 mM NaCl) with a pipette electrode of positive and negative potentials, respectively. In both potentials, the topographical images were successfully obtained. Both images show same topographical structures. The hopping mode of SICM can obtain high-resolution topographic image of cultured cells with minimal deformation in liquid conditions. In general, SICM can image samples by detecting the current drop due to increase the aperture resistance as the pipette aperture edge is approached in the vicinity of the sample surface. In this principle in SICM imaging, it might be possible to image the samples with applying both positive and negative potential. However, in case of dealing with strongly charged samples, it is actually difficult to obtain topographical images because the ion current is strongly influenced in the vicinity of the charged surface.

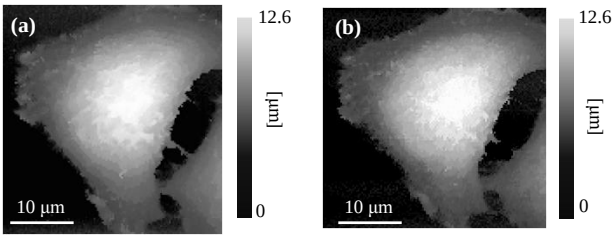

Fig. 3 SICM images of topographic data of the HeLa cell. (a) Image obtained with a pipette electrode of positive potential. (b) Image obtained with a pipette electrode of negative potential.

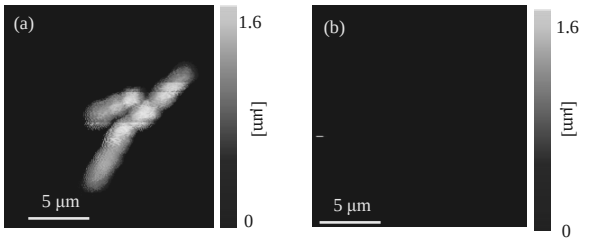

Fig. 4 SICM images of topographic data of the chromosome. (a) Image obtained with a pipette electrode of positive potential. (b) Image obtained with a pipette electrode of negative potential.

Figure 4 shows the topographic data of the chromosome of Indian muntjac. In general, chromosome surface is strongly charged negatively in buffer solution. Thus it is suitable to investigate the influence of the charged effect on SICM imaging. When positive potential was applied to the pipette electrode, the topography of chromosome could be obtained. On the other hand, when negative potential was applied to the pipette electrode, it was difficult to obtain the topography of chromosome. It is considered that the electric charge on the chromosome surface influences the ion current detected by SICM.

3.2 Approach curve

To investigate the difference between the imaging processes under negative and positive potentials, we obtained approach curves indicating how the ion current depends on the distance from the sample surface. Figure 5(a) shows the approach curves obtained on the HeLa cell by applying a positive potential (+0.1 V) and a negative potential (-0.1V) to the nanopipette electrode in the PBS with physiological salt concentration (150 mM). As shown in the figure, in both cases of positive and negative potentials, the ion currents decreased monotonically as the pipette approached the sample surface, even the slight difference between the both curves were recognized. Thus, the reductions of the current could be detected when the pipette edge came close to the surface under each negative and positive potential in hopping mode SICM imaging. On the other hand, on the chromosome surface, the significant difference appeared between the approach curves obtained under the positive and negative potentials as shown in Fig. 5(b). With respect to the approach curve obtained under the positive potential of the pipette electrode, the current decreased as the pipette approached to the sample. However, under the negative potential of the pipette electrode, the current increased as the pipette approached to the sample, and then it dropped suddenly. Therefore, the chromosome image obtained under the negative pipette potential should be significantly different from that obtained under the positive pipette potential, as shown in Fig. 4(a) and (b). On the strongly charged sample surface such chromosome, an excess concentration of cation is likely due to double-layers formed on the surface. Thus, the cation flowing in the pipette aperture

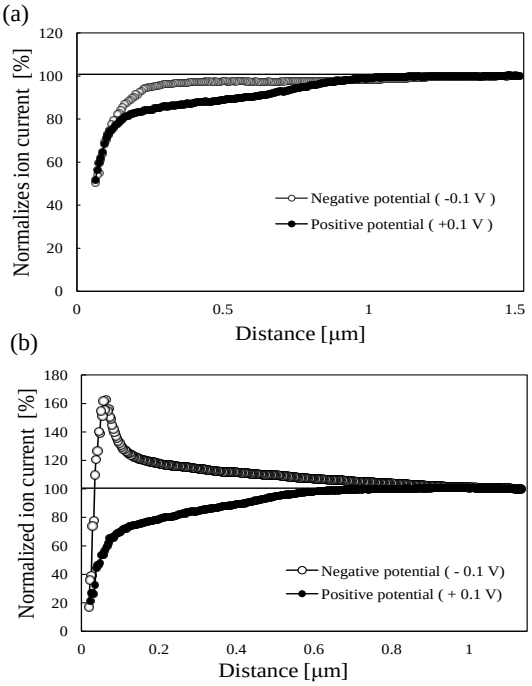

Fig. 5 Approach curves of current behavior as a function of nanopipette-surface distance. (a) Approach curve on the HeLa cell. (b) Approach curve on the chromosome

would be increased as the pipette edge is positioned in the vicinity of the chromosome surface under negative potential, as s result, the topography of the chromosome cannot be imaged in hopping mode because of no detection of the ion current reduction.

3.3 Current-voltage curve

In order to investigation the influence of the charged surface on SICM imaging, current–voltage curve was acquired. Figure 6 show the current voltage curves obtained with the nanopipette positioned on the chromosome surface. The current voltage curves were obtained as the pipette edge was positioned far away from the surface and in the vicinity of the surface of 30% dropped point of the current. In general, the absolute current value detected under positive potential is smaller than that detected under negative potential because of the non-linear rectification behavior of the charges pipette [19]. For the ion current obtained on the chromosome, even the reduction of the current by pipette approaching

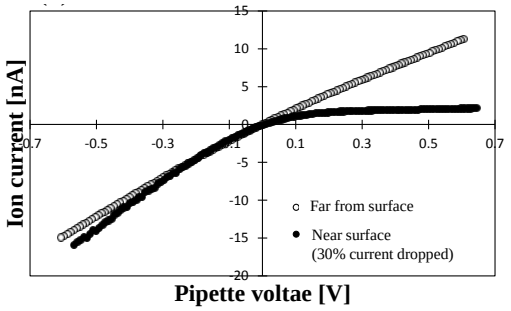

Fig. 6 Current-voltage curves obtained on the chromosome

could be confirmed under positive potential, the increase of the current by pipette approaching was seen under negative potential. As describe above, the effect of the excess concentration of cation due to double-layers on the strongly negative charged surface of the chromosome induces the increase of the ion current under negative potential. Thus, for SICM imaging of the chromosome, the pipette approaching in hopping mode cannot stop on the surface of the chromosome, and finally reaches at the substrate until the current is reduced by occluding of the aperture with the substrate. Therefore as for SICM imaging of the sample in liquid, ion distribution and structure on the charged surface of samples should be considered.

4. Conclusion

We investigate the influence of charge conditions of sample surfaces on imaging using SICM. For SICM imaging of biological samples, it should be considered that the ion current detected with a nanopipette electrode is significantly influenced by charged condition of the sample surface in liquid. On the strongly negative charged surface of the chromosome of Indian Muntjac, it was difficult to obtain the topographic image under negative bias potential of the nanopipette electrode. As for the current behavior as a function of the nanopipette approaching to the sample surface under negative bias potential, the current was increased in the vicinity of the chromosome surface due to the excess concentration of cation on the strongly negative charged surface of the chromosome. Thus, in hopping mode of SICM imaging, it was difficult to image the topography because the ion current reduction was not detected in the pipette approaching. Therefore we should consider the non-linearity behavior of the ion current in the approaching process on charged samples for SICM imaging.

Acknowledgements

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