Coating the Outer Surface of Glass Nanopipette with Chlorobenzene-Terminated Polysiloxane*

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A method of outer surface modification of glass nanopipette with chlorobenzene-terminated organopolysiloxane has been developed. Scanning electron microscope images and microscopic Raman spectra revealed the efficacy of the coating. Energy dispersive X-ray spectra showed not only the materials adsorbed on the nanopipette but also the change in stoichiometry of the bulk glass resulting from the fabrication process of the nanopipette with a laser puller. The coating method is easy to treat and can be used for various applications such as the prevention of carbon contamination from the materials during the injection to biomaterials such as living cells.

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I. INTRODUCTION

Glass nanopipettes have been used for detecting and injecting materials. They can be used in various environments such as in vacuum, air, and liquid [1]. However, a problem lies on the usage of nanopipettes with heavy duties such as injection to sticky cells; the pipettes are contaminated by inserted cells and usually only less than ten times usage is available for the insertion [2, 3].

Chlorobenzene as a cleaning solvent is widely used in the field of silicon device technology [4]. Burkhard discovered that liquid organopolysiloxanes containing the structural unit in the chain and having terminal silicon atoms to each of which are attached three methyl groups when employed for certain purposes, particularly as lubricants, have improved properties over other liquid polysiloxanes, e.g., liquid methylpolysiloxanes, employed in the same application [5]. We have focused on the use of polysiloxane with chlorobenzene functions for the surface modification of the glass nanopipette. Although this kind of coating method has been used for glass plates, coating of glass nanopipette with chlorinated organopolysiloxanes has not been reported yet so that the effectiveness was unknown for the application to the cell injection. Moreover, just coating the nanopipette by immersing into the polymer solution results in the adsorption of the polymer inside the pipette, which might cause the contamination of injecting materials with the polymer.

In this paper, we have developed a method to modify the outer glass nanopipette surface with chlorobenzene termination using a chlorinated polymer. The developed method can avoid the adsorption of the coating polymer inside the nanopipette that might cause the problem of insertion and detection of the materials through the nanopipette. We have investigated the coated and uncoated nanopipettes, before and after using for insertions to materials, with scanning electron microscopy (SEM), Microscopic (spatial resolution of ca. 350 nm) Raman spectroscopy, energy dispersive X-ray spectroscopy (EDX), and electron probe microanalysis (EPMA). The developed method provides an easy way of coating compared with the other methods such as graphene-coating [6].

II. EXPERIMENTAL METHOD

Boron silicate glass nanopipettes with the diameter of ca. 500 nm inner diameter (about 1 μm outer diameter) were fabricated with a puller (Sutter P-2000) from boron silicate glass tubes (Sutter BF100-50-10) [7]. The typical top outer diameter was 800 nm (ca. 400 nm of the inner diameter).

Figure 1 shows how to deposit chlorobenzene-
FIG. 1. Recipe to coat a glass nanopipette with chlorobenzene-terminated polysiloxane only at the outer surface, avoiding the adsorption of the polysiloxane in the nanopipette. Water was filled in the nanopipette prior to the dipping in the nanopipette into the heptane solution of the polysiloxane. Then the nanopipettes were dipped in the heptane solution of chlorobenzene-terminated polysiloxane, and rinsed with ethanol to remove excess polymer on the nanopipette surface. For the details on the recipe, see the text.

FIG. 2. Scanning electron microscope images of nanopipette after the injection to ES cells eight times; uncoated nanopipette (upper) and coated one with chlorobenzene-terminated polysiloxane (lower). Platinum metals were deposited on the pipettes for the observation.

terminated polysiloxane only onto the outer surface of glass nanopipettes. First, the top of nanopipettes were dipped in water for 20 seconds to fill in the water into the nanopipettes from the top. It is noted that filling in water from the proximal end of the nanopipettes does not work well to fill in water to the distal end of the nanopipettes. Second, the nanopipettes were dipped in the heptane solution of chlorobenzene-terminated polysiloxane (Sigma-Aldrich Sigmacote®) for ten seconds, and rinsed with ethanol for a few seconds to remove excess polymer on the nanopipette surface. It is noted that too much rinsing results in the penetration of polysiloxane in the nanopipette. After rinsing, the nanopipettes were kept in a water-filled bottle with a lid to keep the water in the nanopipettes until using.

For the comparison of the coated and uncoated nanopipettes against contamination, the nanopipettes were inserted into mouse embryonic stem (ES) cells EB3, a clone of feeder-free mouse ES cells. We chose ES cells in this study because they have stickier cell membrane than the other cells such as HeLa cells [8]. The EB3 ES cells were provided by Dr. Hitoshi Niwa (RIKEN) [9, 10], and cultured at 37°C in the absence of feeder cells in GMEM (Sigma, St Louis, MO, USA) supplemented with 10% fetal calf serum, 1 mM sodium pyruvate, 10–4 M

FIG. 3. Raman spectra at the top of the used nanopipettes; uncoated nanopipette (a) and chlorobenzene-terminated polysiloxane-coated nanopipette (b). Before using (upper) and After using; eight times injection into ES cells (lower). The small dip at 1200 cm⁻¹ is due to the defect in spectrometer grating, and the peak at 1556 cm⁻¹ is from the graphite formed on the nanopipette.
2-mercaptoethanol, 1 × non-essential amino acids, and 1,000 U LIF/ml on gelatin-coated dishes [11]. A cell suspension containing 5,000–10,000 ES cells was added to the culture dish and incubated at 37°C. These procedures [7] were conducted at Tokyo University of Agriculture and Technology. We checked the reuse ability; we could use the coated nanopipette for the cell injection more than twenty times while less than six times for the uncoated nanopipettes.

The nanopipettes were observed with SEM (Hitachi S-4800) and microscopic Raman spectrometer (Tokyo Instruments Nanofinder 30) with the special resolution of 350 nm (SPEX 500M) at Central Analytical Facility of IMRAM in Tohoku University. The wavelength of the Ar laser source was 532 nm. The measurements of EDX and EPMA were conducted with the field emission scanning electron microscope (Hitachi S-5200) and X-ray spectrometer (EDAX Genesis XM2) at N-BARD in Hiroshima University.

III. RESULTS AND DISCUSSIONS

Scanning electron microscope images clearly shows the effectiveness of the coating onto glass nanopipettes, as shown in Fig. 2. Although the observed nanopipettes were deposited with platinum metals using a sputter to prevent the observed surfaces from the charge-up due to the irradiation of the electron beam, the rough surface morphology was observed on the uncoated nanopipette, whereas the fairly smooth surface morphology was observed on the coated nanopipette. We observed twelve uncoated and twelve coated nanopipettes and found the difference in morphology between coated and uncoated nanopipettes. The difference in the morphology on the SEM observation was also confirmed with the nanopipette samples deposited using an anti-static spray [12] though the resolution of the obtained images was not enough compared with the SEM images shown in Fig. 2, due to the charge-up of the top part of the glass nanopipette.

The effectiveness of the coating was also confirmed with the measurements of the microscopic Raman spectra. Upper column in Fig. 3 shows the spectra of the nanopipettes before using and the lower column shows that of used ones (insertion to ES cells eight times), for the uncoated nanopipettes (a) and the coated nanopipettes (b). The upper column spectra show no contamination on the pipette surface before using. On the used nanopipettes, the observed bands at 1365 cm\(^{-1}\) assigned to sp\(^3\) carbon and 1600 cm\(^{-1}\) assigned to sp\(^2\) carbon, respectively [13], attributed to the carbon contamination from the ES cells, were observed in the spectrum (a) whereas such bands were not observed in the spectrum (b), indicating the effectiveness of the nanopipette coating with chlorobenzene-terminated polysiloxane.

On the nanopipettes before using, we could not observe the difference between coated and uncoated nanopipette in the Raman spectra, as shown in the upper column in Fig. 3, which may be due to the reason that the chlorobenzene-terminated polysiloxane formed almost monolayer at the top of the glass nanopipettes and the amount is too small to observe in this experiment. We also measured the range at 0–1000 cm\(^{-1}\) and no peak assigned to C–Cl stretching was observed. The peaks indicated with an asterisk (*) in the Raman spectra shown in Fig. 3 are assigned to the graphite on the nanopipettes. The graphite is formed due to the heat to the nanopipette during the fabrication process with a laser puller, or during the Raman observation with a strong laser beam. To solve the problem of the heat dur-
FIG. 5. Scanning electron microscope image at the shank of the uncoated nanopipette after the injection to ES cells eight times (upper left), the EDX spectrum (upper right), and the electron probe micro analysis images of the same area for the each element (O, Si, C, P, and Na). Imposed figure in the EDX spectrum: cross sectional image profile at the line indicated in the SEM image shown at the upper right I the imposed figure, for the each element of C, Si, O, Ca, P, and Na.

FIG. 6. Energy dispersive X-ray spectra of the uncoated nanopipette after the injection to ES cells eight times, at the bulk (blue), shank (red), and top (green), respectively, as shown in the schematic drawing imposed in the figure. The peak intensity of the spectra was normalized with the peak assigned to Si. The values of the O/Si intensity ratio were shown in the table imposed in the figure.

An interesting finding on the O/Si stoichiometry was obtained from the EDX spectra of the nanopipettes observed at the top, shank, and bulk, as shown in Fig. 6. The top part is a quartz-like stoichiometry (SiO$_2$) whereas the bulk is an amorphous glass (SiO). The intermediate shank part has an oxygen-poor stoichiometry (Si$_2$O) since the oxygen atoms were taken to the top part of the pipette during the fabrication process [16]. It is known that the oxygen-poor stoichiometry in glass can be produced with KrF excimer laser [17], Ar$_2$ excimer laser [18], and synchrotron radiation [19]. The puller used in this study can produce the temperature more than the melting temperature of boron silicate. Further studies are necessary to investigate the stoichiometry of the glass pipette at each point, including the mechanism to generate the different stoichiometry at the top, shank, and the intermediate points.

In order to confirm that the inside of the nanopipette was not coated, the following experiment was conducted. After the preparation of the coated nanopipettes with
the same recipe shown in Fig. 1, the top of the coated nanopipettes were smashed and observed the smashed pieces with EPMA and EDX, as shown in Fig. 7. Whether the smashed pieces were outer surface or inner surface could be distinguished from the curvature of the pieces. Although the signal assigned to chlorine atom in coating polymer could not be observed due to the irradiation of the electron beam in vacuum resulting in the evaporation, the signal assigned to carbon K\textsubscript{L} line from the outer nanopipette surface was larger than that from the inner nanopipette surface, which could be the evidence that the inside of the nanopipette was not coated. Further investigation is necessary what extent the coating polymer penetrate into the inside of the nanopipette during the coating process.

IV. CONCLUSION

We have developed a method to coat only the outer surface of glass nanopipettes. The water filled in the inner nanopipettes before the coating process prevent the inner wall from depositing the polysiloxane in heptane solution. We have applied the coated nanopipettes to the insertion into ES cells and confirmed the effectiveness of the coating. The developed method will increase the sustainability including the viability of injected cells because the coated nanopipettes will decrease the damage to the injected cells.

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[1] T. Takami, B. H. Park, and T. Kawai, Nano Convergence 1, 17 (2014).
[2] H. Matsuoka, M. Saito and H. Funabashi, in M. Kallos (Ed.), Embryonic Stem Cells - Basic Biology to Bioengineering (InTech, 2011).
[3] H. Matsuoka, private communication.
[4] M. Rossberg, W. Lendle, G. Pfeiderer, A. Tögel, E. L. Dreher, E. Langer, H. Rassaerts, P. Kleinschmidt, H. Strack, R. Cook, U. Beck, K. A. Lipper, T. R. Torkelson, E. Löser, K. K. Beutel, and T. Mann, in Ullmann’s Encyclopedia of Industrial Chemistry (Wiley Online Library, 2006), DOI: 10.1002/14356007.a06_233.pub2.
[5] C. A. Burkhard, US Patent, 2689859A (1950).
[6] D. Choi and D. S. Kim, Langmuir 30, 6644 (2014).
[7] H. Matsuoka, S. Shimoda, M. Ozaki, H. Mizukami, M. Shibusawa, Y. Yamada, and M. Saito, Biotechnol. Lett. 29, 341 (2007).
[8] H. Matsuoka, T. Komazaki, Y. Mukai, M. Shibusawa, H. Akane, A. Chaki, N. Uetake, and M. Saito, J. Biotechnol. 116, 185 (2005).
[9] H. Niwa, S. Masui, I. Chambers, A. G. Smith, and J. Miyazaki, Mol. Cell Biol. 22, 1526 (2002).
[10] K. Ogawa, H. Matsu, S. Ohitsuka, and H. Niwa, Genes. Cells 9, 471 (2004).
[11] A. G. Smith, J. Tissue Cult. Methods 13, 89 (1991).
[12] Z. E. Long, S. Imashuku, and J. Kawai, Bunseki Kagaku (in Japanese) 62, 155 (2013).
[13] A. C. Ferrari and J. Robertson, Phys. Rev. B 61, 14095 (2000).
[14] H. Nakao, S. Tokonami, T. Hamada, H. Shiigi, T. Nagaoa, F. Iwata, and Y. Takeda, Nanoscale 4, 6814 (2012).
[15] We carefully treated the boron silicate glass tubes, the resource of the nanopipette. The tubes were kept in a large test tube with a lid, and we used rubber gloves for the operation. However, the Raman peak showing the graphite contamination appeared. Even if we wiped the glass tubes with ethanol and water before making the nanopipettes using Sutter P-2000, the graphite peak still remained. According to the knowledge on surface treatment in ultra-high vacuum (UHV), the carbon contamination can be removed by the annealing the resource glass tubes in high vacuum, which is a common treatment for the researchers working on UHV though we do not have such a chamber in usual laboratories in biology.
[16] The shank part is not identical condition comparing to the bulk and top of the pipette; the angle is not the same for EDX analysis. However, it did not affect the stoichiometric ratio of silicon and oxygen if the component ratio was homogeneous at the irradiated area of the electron beam. Future studies will make it clear the exact stoichiometry at each point.
[17] C. Fiori and R. A. B. Devine, Appl. Phys. Lett. 47, 361 (1985).
[18] K. Kurosawa, Y. Takigawa, W. Sasaki, M. Katto, and Y. Inoue, Jpn. J. Appl. Phys. 30, 3219 (1991).
[19] H. Akazawa, J. Takahashi, Y. Utsumi, I. Kawashima, and T. Urisu, Appl. Phys. Lett. 60, 974 (1992).