Nanopipette with a lipid nanotube as nanochannel

doi: 10.1109/NANO.2007.4601400

Proceedings of the 7th IEEE
International Conference on Nanotechnology
August 2 - 5, 2007, Hong Kong

Nanopipette with a Lipid Nanotube as Nanochannel

Kousuke Nogawa[1*], *Student Member*, *IEEE*, Yusuke Tagawa[2], Masahiro Nakajima[1], *Member, IEEE*, Fumihito Arai[3], *Member, IEEE*, Toshimi Shimizu[4], Shoko Kamiya[4], and Toshio Fukuda[1], *Fellow, IEEE*

[1]*Department of Micro-Nano Systems Engineering, Nagoya University, Japan*
[2]*Department of Mechanical Science and Engineering, Nagoya University, Japan*
[3]*Department of Bioengineering and Robotics, Tohoku University, Japan*
[4]*Nanoarchitectonics Research Center, National Institute of Advanced Industrial Science and Technology, Japan*

***Abstract* — Single cell analysis gets a lot of attentions to reveal the unknown biological aspects of individual cells. To analyze properties of a single cell, local environmental control is desired. We propose a novel type of nanopipette where a lipid nanotube (LNT) as a nanochannel is attached to apply the minimal changes to the environment. LNTs have hollow cylindrical nanostructures consisting of lipid bilayer membranes and their outer and inner surfaces are hydrophilic. Fabrication process of the LNT nanopipette includes two main parts; to pick up an LNT and to seal the interspace between it and glass micropipette. The fluorescent solution was spouted from the fabricated LNT nanopipette. The nanopipette is effective to local environmental control as an end-effecter for biological applications.**

Keywords — ***Lipid nanotubes, Local environmental control, Micromanipulation, Nanopipette, Single cell analysis***

I. Introduction

Single cell analysis gets a lot of attentions to reveal the specified and localized biological information of individual cells. To analyze the properties of single cell, the local environmental control technique, which is the technique to change and sense the local environment around single cells, is desired. The probe type devices, like glass micropipettes, have high operationality and workability when they are used as an end-effecter of micro manipulator. And they can change the environment near or inside a single cell. Recently, the submicron-scale local environment has been controlled by use of nanomaterials and nano-tools fabricated with the improved microfabrication techniques.

Nanopipette, which is a pipette with nano-scale ejection hole, is considered to be an effective tool for the local environmental control technique. Some fabrication methods of nanopipettes have been already developed. Mani *et al.* proposed the tapered carbon nanowhiskers with hollowness as pipettes [1]. The unique nanowhiskers might be a powerful tool to form pointed tips which act as nanopipettes. It is needed to show that the hollowness act as nanochannel for liquid. Freedman *et al.* proposed carbon nanotube (CNT) tipped pipettes assembled magnetically and fixed with an optical adhesive [2]. The fabrication process using magnetic CNT is unique. There is possibility that a number of CNTs attach on the tip of pipette and form multiple ejection holes. The suction of a solution was demonstrated, but the spout has not been presented. Kometani *et al.* presented the Focused-Ion-Beam Chemical Vapor Deposition (FIB-CVD) fabrication of a nanopipette by depositing nozzle nanostructure on the tip of glass micropipette [3]. The possibility is shown to fabricate various types of nanopipettes on conventional micropipettes. To fabricate reliable and smooth nanopipettes, a plenty of the operation time and an advanced 3-dimensional FIB-CVD system are needed. Clarke *et al.* fabricated a nanopipette by the commercialized puller with borosilicate glass capillary [4]. The fabrication with the puller is relatively easy and reliable. However the shape of tapered part can not be precisely controlled and its tip is quite thin. These are causes of the thermal vibration at the tip and a disadvantage in low mechanical strength. As these results, nanopipette leading in shape, machinability and locality is desired to spout the liquid.

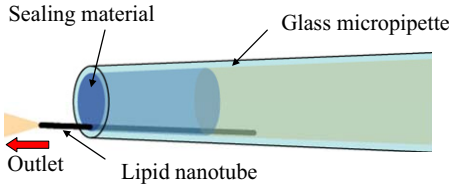

Figure 1. A schematic of LNT nanopipette.

In this paper, we propose a nanopipette with a lipid nanotube (LNT) as a nanochannel. The LNT nanopipettes are examined of the spout of the fluorescent solutions by applying DC voltage.

II. Design of LNT Nanopipette

A schematic of LNT nanopipette is shown in Fig. 1. Single LNT is fixed at the tip of a glass micropipette and the interspace between the LNT and the glass micropipette is sealed with sealing material to use the LNT as a nanochannel.

A. Lipid Nanotube

Hollow cylindrical structures of lipid molecules appeared in a series of aggregate morphologies with high axial ratios. The first reports of the formation of LNTs from bilayer-forming amphiphiles came independently and almost simultaneously from three research groups in the United States and Japan [5-7]. It should be noted that these nanotubes were reported about seven years before Iijima discovered the existence of multiwall carbon nanotubes [8,9].

The LNTs are made of the lipids which have both hydrophilic and hydrophobic group in monomolecular by self-assembly, and have hollow cylindrical nano structures

This work was partially supported by MEXT KAKENHI (17076004).
**Contacting Author: Kousuke Nogawa is with the Department of Micro-Nano Systems Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, 464-8603, Japan (phone: +81-52-789-4481; fax: +81-52-789-3115; e-mail: nogawa@robo.mein.nagoya-u.ac.jp).*

consisting of lipid bilayer membranes. The outer and inner surfaces of the LNTs are hydrophilic [8,10]. The mechanical characteristics of LNTs have been measured, for example Young's modulus is ~720 MPa [8,11]. Ordinarily, LNTs are preserved in dry condition or dispersed in water. (In this paper, LNTs dispersed in water were used.) The inner/outer diameters of the LNTs used in this paper were each ~40 nm/ ~400 nm, and their length were in the range of 10~100 μm.

As previous works, the fluorescent solution has been confined inside LNTs [12]. We thought that LNT can be used as a nanochannel based on this instance. And LNTs have suitable dimensions to be observed during fabrication and application by optical microscope. When we use other nanotubes, for example carbon nanotubes (CNTs), the manipulation technique inside electron microscopes are needed to observe them for assembly [13,14]. Hence, in this study, we selected LNTs as the nanochannel of nanopipettes.

B. Sealing Material

If there is interspace between the LNT and the glass micropipette, it is impossible to carry out the function as nanopipette, because of the leakage of the solution. The sealing material is used to fill the interspace between the LNT and the glass micropipette. The sealing material is desired to have high adhesiveness to the glass, low shrinkage during curing and high waterproof property.

In this study, photo-crosslinkable resin TB3036 (Three Bond, Japan) was used. Photo-crosslinkable resin is a resin which is caused radical polymerization or cationic polymerization by the ultraviolet rays irradiation of the wavelength of 200~400 nm. Especially, TB3036 is cured at short times by the ultraviolet rays irradiation, and have the features such as low shrinkage during curing (4.7%), low water absorption coefficient (1.2%) and high adhesiveness to the plastic, glass and metal. Hence, TB3036 is suitable for sealing material.

C. Glass Micropipette

The glass micropipette is fabricated by pulling the borosilicate glass tube (Outer/inner diameters are each 1 mm/0.6 mm; GD-1, Narishige, Japan) with the puller (PC-10, Narishige, Japan). Its diameter can be controlled by the heating power and load. (Generally the minimum inner diameter of the tip of the glass micropipette is ~1 μm.)

If the inner diameter of the glass micropipette is too small, it is difficult to insert an LNT into it because of the adhesion to its inner wall by van der Waals force. Therefore, in this study, glass micropipette with ~2 μm inner diameter was used for picking up of LNT. On the other hand, glass micropipette with smaller diameter is necessary for the injection of photo-crosslinkable resin into the glass micropipette. Hence, the glass micropipette with ~1 μm inner diameter was used for filling photo-crosslinkable resin.

D. Method of Spout of Liquid

Generally, with a nano-scale channel, it is difficult to pass and spurt the fluid by pressure, because the influence of

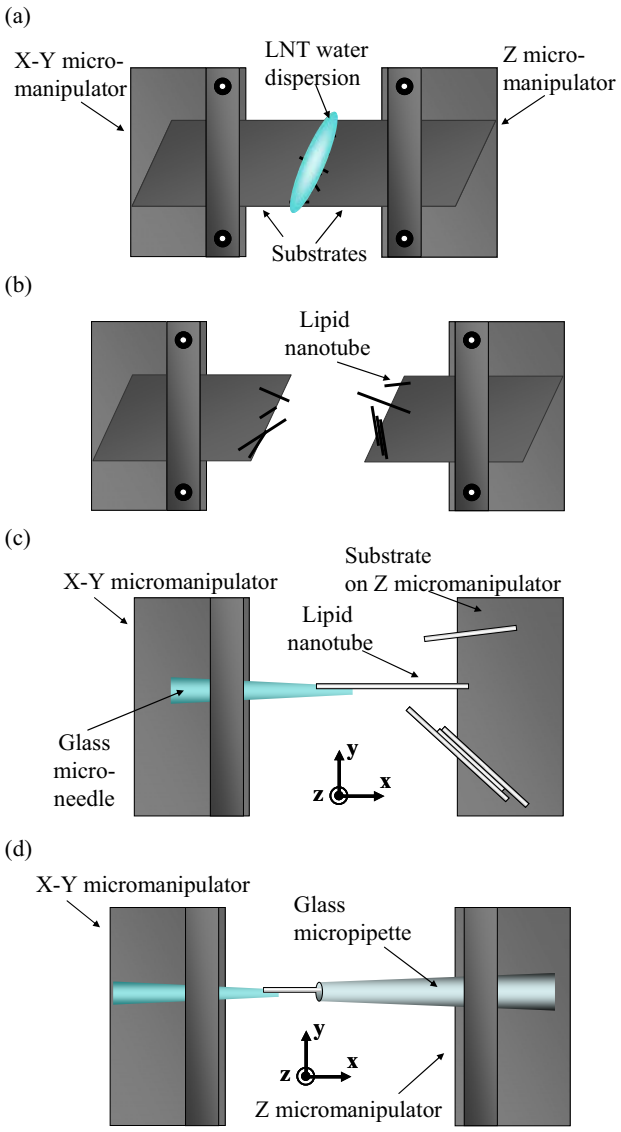

Figure 2. Schematics of picking up process. (a) Positioning of substrates and droplet of LNT water dispersion. (b) Separating substrates with each other. (c) Picking up of vertically arranged LNT by glass micro-needle. (d) Picking up of LNT inside glass micropipette from the needle.

frictional force increases [15]. To overcome such a difficulty, Ying *et al.* spouted the fluids from the borosilicate glass nanopipette with electric migration force by applying DC voltage between solution inside of pipette and solution in the bath [16]. We applied the DC voltage to spout the fluorescent solution from the LNT nanopipette with basically same method.

III. Fabrication Process of LNT Nanopipette

Fabrication process of the LNT nanopipette is consisted of two processes; picking up and sealing processes.

A. Picking up Process

As picking up process, a single LNT is picked up inside glass micropipette by use of micromanipulator. Schematics of picking up process are shown in Fig. 2.

To pick up a single LNT, it is necessary to arrange LNT with vertically and singularly cantilevering about 10 μm from the edge of the substrate. If LNTs form bundles in dry condition, LNTs bind each other by mainly surface tension of water because of their hydrophilicity. Moreover the bundles of LNTs are hard to be separated. When LNT nanopipette is made of a bundle of LNTs, some ejection holes will be formed. It is not suitable for ultralow volume spout. Hence we picked up the single LNT by micromanipulation.

First, two substrates were positioned several μm distance from each other by X-Y micromanipulator (As the X-Y micromanipulator, XY axis stage (TSD-252S, Sigma Koki, Japan) is used. Its work space is ±3 mm). Then, LNT water dispersion was dropped onto the gap of these substrates (Fig. 2 (a)). Two substrates were separated with each other after evaporating of the dispersion. The LNTs are arranged at random on the edge of the substrates (Fig. 2 (b)). Single LNT protruded vertically was picked up on the glass micro-needle (outer diameter of the tip: ~1 μm) by use of X-Y and Z micromanipulators (As the Z micromanipulator, Z axis stage (TSD-253, Sigma Koki, Japan) is used. Its work space is ±3 mm) under monitoring with stereoscopic microscope (VH-Z250, Keyence, Japan) (Fig. 2 (c)). (The glass micro-needle is made by pulling borosilicate glass rod (Outer diameter: 1 mm, G-1000, Narishige, Japan).) The LNT is picked up on the glass micro-needle by mainly van der Waals force. Then, the LNT was picked up inside glass micropipette (Inner diameter is ~2 μm.) from the needle under monitoring with the stereoscopic microscope (Fig. 2 (d)). The reason why an LNT was picked up with two-step approach is that LNT often breaks if it is picked up directly by glass micropipette. Because, long contact segment of LNT and the substrate cannot set apart easily so LNT is stressed at the tip of glass micropipette. To verify the insertion, the LNT was pushed sidearm by the glass micro-needle. If the LNT was not inserted, it was moved or rotated easily. LNT must be inserted into the glass micropipette deep enough not to be filled up with photo-crosslinkable resin in the next sealing process. In this experiment, LNT was inserted ~5 μm into glass micropipette.

B. Sealing Process

As sealing process, the interspace between the LNT and the glass micropipette is sealed with photo-crosslinkable resin. Schematics of sealing process are shown in Fig. 3.

Photo-crosslinkable resin was soaked inside another glass micropipette (inner diameter of the tip: ~1 μm) by capillarity (Fig.3 (a)). Its tip was positioned near the tip of the glass micropipette which picked up an LNT by X-Y micromanipulator under monitoring with the stereoscopic microscope (Fig.3 (b)). Then, the resin was pushed out by pressure using a syringe manually. The pushed out resin was soaked into the interspace between the LNT and the glass micropipette by capillarity on contact with the tip of the glass micropipette which picked up an LNT (Fig.3 (c)). When the interspace between the LNT and the glass micropipette was filled with the resin, the pressure was released. In this step, the both ends of the LNT must not be filled up with the resin. Then, the resin was cured by 5 seconds exposure of UV light (Fig.3 (d)). (The light source was short arc mercury lamp (USH-1030L, Ushio, Japan) and UV light was irradiated through mirror unit (U-MWU2, Olympus, Japan) and objective lens (LUCPLFLN 60×, Olympus, Japan) under monitoring with inverted optical microscope (IX71, Olympus, Japan).)

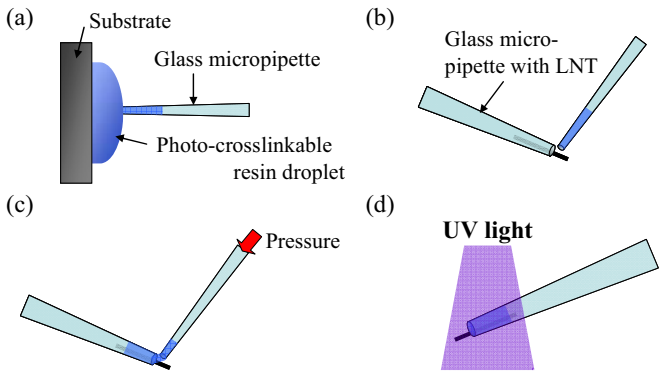

Figure 3. Schematics of sealing process. (a) Soaking of photo-crosslinkable resin by capillarity. (b) Bringing the pipettes close to each other. (c) Pushing out the resin from the right pipette by pressure. (d) Exposure of UV light to cure the photo-crosslinkable resin.

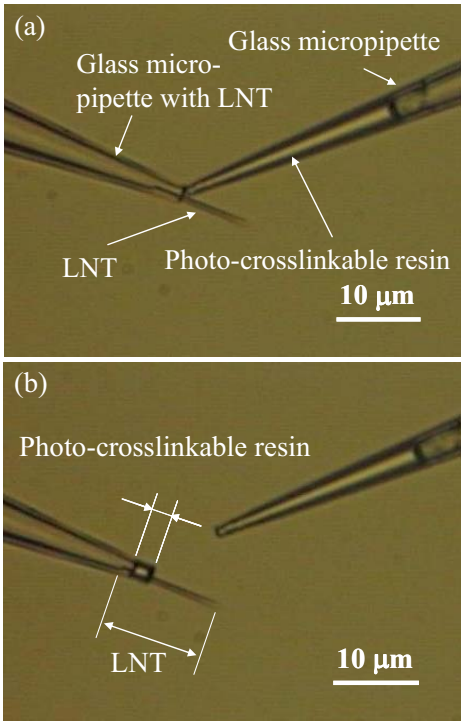

Figure 4. Experimental results of sealing process. (a) Injection of the photo-crosslinkable resin into the left pipette with LNT from the right pipette. (b) After injection of the resin.

Total fabrication time of picking up and sealing processes is approximately two hours.

IV. Experimental Results and Discussions

A. Fabrication of LNT Nanopipette

Optical microscope images of experimental results of sealing process are shown in Fig. 4. The photo-crosslinkable

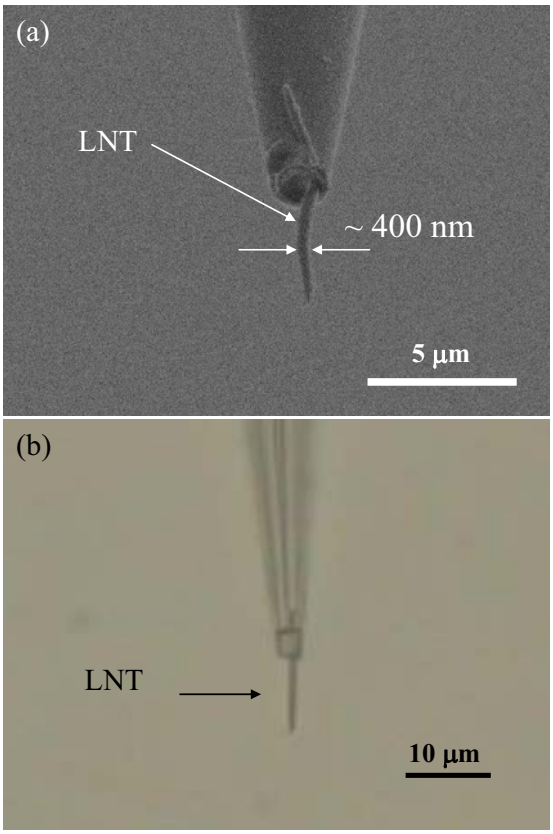

Figure 5. A fabricated LNT nanopipette. (a) SEM image. (b) Inverted optical microscope image.

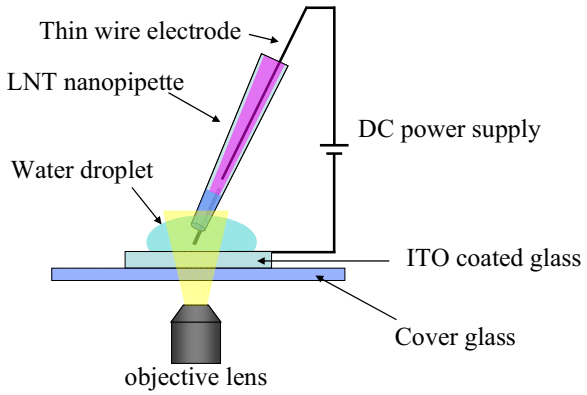

Figure 6. A Schematic of experimental setup for the spout of the fluorescent solution.

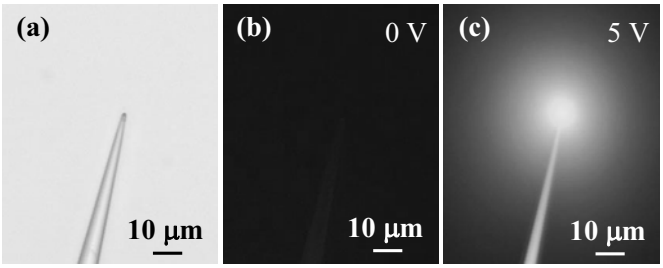

Figure 7. Experimental results of the spout of the fluorescent solution from glass micropipette (inner diameter; ~1 mm). (a) Bright field image before applying a voltage. (b)(c) Dark field images at the DC voltages of (b) 0 V and (c) 5 V.

resin was soaked into the glass micropipette which picked up an LNT by capillarity (Fig. 4 (b)).

A fabricated LNT nanopipette is shown in Fig. 5. Fig. 5 (a) is SEM image and Fig. 5 (b) is inverted optical microscope image of the same LNT nanopipette. This LNT nanopipette has the micro-scale glass tip with nano-scale ejection hole. It is expected enough mechanical stiffness to be inserted into biological samples. (In this case, the protruded part of the LNT from the resin has to be chemically melted.)

B. Experimental Setup for Spout of Fluorescent Solution

We checked the spout of fluorescent solution from the LNT nanopipette by dark field fluorescent image of inverted optical microscope (IX71, Olympus, Japan). The schematic of experimental setup is shown in Fig. 6.

At first, LNT nanopipette was enclosed the fluorescent solution by capillarity from the open end of glass micropipette, and then its tip was put in pure water bath. The fluorescent solution was Rhodamine 6G aqueous solution (MP Biomedicals, Inc., Germany; ~4.5×10^{-3} wt%). The negative electrode was connected to the ITO (Indium Tin Oxide; it is a conductive transparent electrode.) coated glass. And the positive electrode was connected to the thin conductive wire inserted into LNT nanopipette. The distance between the tip of LNT nanopipette and the ITO coated glass was approximately 1 mm. And the linear distance between the tip of the thin conductive wire and the ITO coated glass was approximately 2 mm. DC voltage was applied between these two electrodes (DC power supply; EX-375U2, Takasago, Japan. Maximum voltage: 526 V).

C. Pre-experiment using Glass Micropipette

As pre-experiment, the fluorescent solution was spouted from the glass micropipette (Inner diameter is ~1 μm) by the same experimental setup with nanopipette. The bright field image of the glass micropipette is shown in Fig. 7 (a). From the fluorescent image without applying DC voltage, the spout of fluorescent solution was not observed (Fig. 7 (b)). The fluorescent solution started to spout by applying the DC voltage. The dark field fluorescent image at the DC voltage of 5 V is shown in Fig. 7 (c). Additionally, the solution was not spouted from fully sealed glass micropipette with applying the maximum DC voltage.

D. Spout of Fluorescent Solution from LNT Nanopipettes

Then, the spout experiment from the LNT nanopipette was conducted. The bright field image of the LNT nanopipette before experiment is shown in Fig. 8 (a). The DC voltages were increased from 0 V to 526 V (Fig. 8 (b)-(g)). At the DC voltage of 200~300 V, the fluorescent solution started to spout gradually near the end of the LNT nanopipette. The spouting volume increased with multiplying the DC voltage. This result confirmed the spout of solution from the LNT nanopipette, and showed that the spouting volume can control by applied voltage.

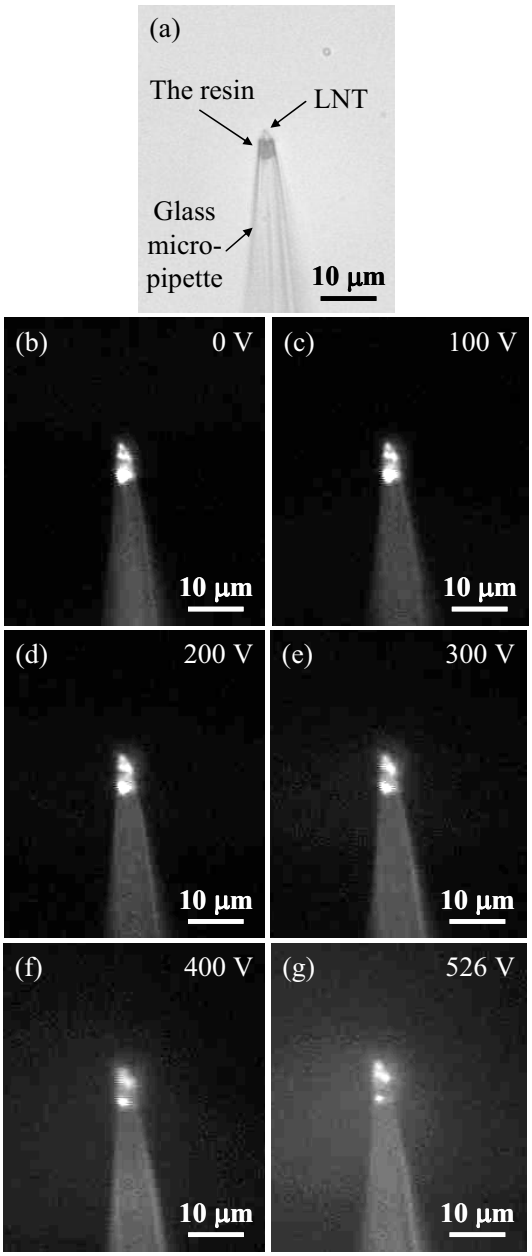

Figure 8. Experimental results of the spout of the fluorescent solution from the LNT nanopipette. (a) Bright field image before applying a voltage. (b)-(g) Dark field images at the DC voltages of (b) 0 V, (c) 100 V, (d) 200 V, (e) 300 V, (f) 400 V and (g) 526 V.

The spouting volume from the LNT nanopipette (Fig. 8) is quite ultralow as compared with the result of glass micropipette (Fig. 7). Therefore it was confirmed that spouting volume can control in ultralow level by use of the LNT nanopipette.

As the future work, we will apply the LNT nanopipette to biological field as a local environmental control tool for the single cell by building it into micromanipulation system under optical microscope. For these applications, some issues, like the conditions of buffer and solution, the diffusion control of the spouted solution, and effective patterning of microelectrodes are considered to be important factors.

V. Conclusion

We proposed and fabricated a novel nanopipette with an LNT as a nanochannel. It was fabricated by picking up an LNT inside a glass micropipette and sealing the interspace with photo-crosslinkable resin. The spout of fluorescent solution was controlled by applied DC voltage. From the florescent images, it was confirmed that the spouting volume from the LNT nanopipette can control in ultralow level compared with glass micropipette.

References

[1] R. C. Mani, X. Li, M. K. Sunkara, and K. Rajan, "Carbon Nanopipettes," *Nano Lett.*, Vol.3, No.5, pp.671-673, 2003.

[2] J. R. Freedman, D. Mattia, G. Korneva, Y. Gogotsi, G. Friedman, and A. K. Fontecchio, "Magnetically Assembled Carbon Nanotube Tipped Pipettes," *Appl. Phys. Lett.*, Vol.90, 103108, 2007.

[3] R. Kometani, T. Morita, K. Watanabe, K. Kanda, Y. Haruyama, T. Kaito, J. Fujita, M. Ishida, Y. Ochiai, and S. Matsui, "Nozzle-Nanostructure Fabrication on Glass Capillary by Focused-Ion-Beam Chemical Vapor Deposition and Etching," *Jpn. J. Appl. Phys.*, Vol.42, pp.4107-4110, 2003.

[4] R. W. Clarke, S. S. White, D. Zhou, L. Ying, and D. Klenerman, "Trapping of Proteins under Physiological Conditions in a Nanopipette," *Angew. Chem. Int. Ed.*, Vol.44, pp.3747 -3750, 2005.

[5] K. Yamada, H. Ihara, T. Ide, T. Fukumoto, and C. Hirayama, "Formation of Helical Super Structure from Single-Walled Bilayers by Amphiphiles with Oligo-L-Glutamic Acid-Head Group," *Chem. Lett.*, Vol.10, pp.1713-1716, 1984.

[6] N. Nakashima, S. Asakuma, J. M. Kim, and T. Kunitake, "Helical Superstructures are Formed from Chiral Ammonium Bilayers," *Chem. Lett.*, Vol.10, pp.1709-1712, 1984.

[7] P. Yager, and P. E. Schoen, "Formation of Tubules by a Polymerizable Surfactant," *Mol. Cryst. Liq. Cryst.*, Vol.106, pp.371-381, 1984.

[8] T. Shimizu, M. Masuda, and H. Minamikawa, "Supramolecular Nanotube Architectures Based on Amphiphilic Molecules," *Chem. Rev.*, Vol.105, pp.1401-1443, 2005.

[9] S. Iijima, "Helical Microtubules of Graphitic Carbon," *Nature*, Vol.354, pp.56-58, 1991.

[10] S. Kamiya, H. Minamikawa, J. H. Jung, B. Yang, M. Masuda, and T. Shimizu, "Molecular Structure of Glucopyranosylamide Lipid and Nanotube Morphology," *Langmuir*, Vol.21, pp.1401-1443, 2005.

[11] H. Frusawa, A. Fukagawa, Y. Ikeda, J. Araki, K. Ito, G. John, T. Shimizu, "Aligning a Single-Lipid Nanotube with Moderate Stiffness," *Angew. Chem. Int. Ed.*, Vol.42, No.1, pp.72-74, 2003.

[12] H. Yui, Y. Guo, K. Koyama, T. Sawada, G. John, B. Yang, M. Masuda, and T. Shimizu, "Local Environment and Property of Water inside the Hollow Cylinder of a Lipid Nanotube," *Langmuir*, Vol.21, pp.721-727, 2005.

[13] M. Nakajima, F. Arai, and T. Fukuda, "In situ Measurement of Young's Modulus of Carbon Nanotube inside TEM through Hybrid Nanorobotic Manipulation System," *IEEE Transactions on Nanotechnology*, Vol.5, No.3, pp.243-248, 2006.

[14] P. Liu, F. Arai, and T. Fukuda, "Cutting of Carbon Nanotubes Assisted with Oxygen Gas inside a Scanning Electron Microscope," *Appl. Phys. Lett.*, Vol.89, No.11, 113104, 2006.

[15] T. M. Truskett, "The Subtleties of Water in Small Spaces," *Proc. Natl. Acad. Sci. U. S. A.*, Vol.100, No.18, pp.10139-10140, 2003.

[16] L. Ying, A. Bruckbauer, D. Zhou, J. Gorelik, A. Shevchuk, M. Lab, Y. Korchevb and D. Klenerman, "The Scanned Nanopipette: a New Tool for High Resolution Bioimaging and Controlled Deposition of Biomolecules," *Phys. Chem. Chem. Phys.*, Vol.7, pp.2859-2866, 2005.