ABSTRACT
We proposed hybrid nanorobot that can be manipulated by optical tweezers and has hybrid structure integrating functional nanomaterials. We employed femtosecond laser exposure for fabrication of the nanorobot with 3D nanometric structure. We succeeded in fabricating nanorobot with carbon nanotubes (CNTs) that has a high photothermal efficiency. Furthermore, we evaluated optical manipulation characteristics of the nanorobot and demonstrated single cell puncture with the nanorobot. We performed single cell puncture by irradiating infrared (IR) laser to CNT and generating heat on that point.

KEYWORDS: anorobot, Carbon anotube, Optical Tweezers, Cell Surgery

INTRODUCTION
Single cell manipulation or surgery are highly required for detailed cell analysis in recent biotechnology. Conventional cell manipulation or surgery are mainly conducted by manual operation of micromechanical manipulators. However, there are some problems such as contamination, low repeatability, low-throughput, or operator skill dependency. Our group has proposed on-chip robot for resolving these problems [1][2]. By integrating microrobot on microfluidic chip, we realized complicated cell manipulation or surgery. These on-chip robot were driven by magnetic attractive force and there was friction between the robot and a substrate. Therefore, positioning accuracy of magnetically driven on-chip robots were not so good (≈ μm) due to the friction. Furthermore, previous on-chip robots has manipulation or surgery probe with the size of a few μm because they are fabricated by standard mask lithography method. It is relatively large for a small somatic cell (≈ 10μm) and has been used only for large cells such as oocytes or aquatic single-celled organisms. Optical tweezers are powerful manipulation method in microfluidic chip because of their high positioning accuracy and frictionless property. However, their manipulation force is week (≈ pN) and it was used only for simple cell manipulation or mechanical stimulation for very soft cell [3].

In this paper, we proposed hybrid nanorobot that can be manipulated by optical tweezers and has hybrid structure with functional nanomaterials. By utilizing various characteristics of nanomaterials, we can create new function of nanorobots. In this study, we succeeded in fabricating a nanorobot with CNTs and a single cell puncture with the nanorobot.

EXPERIMENTAL SYSTEM SETUP
To fabricate nanometric structure of the nanorobot, we employed femtosecond laser exposure that can fabricate nanometric 3D structures. As a light source of the exposure system, ultrashort pulsed titanium-sapphire laser (Coherent Co. Ltd., Chameleon) which wavelength was 780 nm and a pulse width was 140 fs was employed. The laser beam was focused on a sample through a microscope objective lens (Olympus, UPLSAPO 100XO). We employed piezoelectric XYZ stage (Physik Instrumente GmbH Co., P-563) for a positioning of exposed samples. An exposure area of this system was 300 μm x 300 μm in a horizontal plane and 130 μm in a vertical axis. For manipulation of the nanorobot, we employed holographic optical tweezers (HOT) system that had been previously constructed by our group [3]. A Yb fiber laser (IG Photonics Co. Ltd.) of 1064 nm wave length was employed for laser source because IR laser has a relatively lower damage to living cells. The hybrid nanorobot having multi-trap points can be manipulated by HOT. These fabrication and manipulation systems are integrated in identical microscope (Figure 2).
FABRICATION OF HYBRID NANOROBOT

We realized the hybrid nanorobot by sandwiching CNTs by negative photoresist layers. We used a negative photoresist (SU-8, Nippon Kayaku Co. Ltd.) as a material of the nanorobot. Figure 3 shows the fabrication process of the hybrid nanorobot and details are described in followings.

1) Spin coat SU-8 on a glass substrate.
2) Coat CNT (Multi-walled CNT, Sigma-Aldrich Co. LLC.) dispersion on the spin coated SU-8 layer.
3) Spin coat second SU-8 layer over the CNTs.
4) Pattern nanorobot by femtosecond laser exposure.
5) Develop the exposed nanorobot.
6) Package the chip by polydimethylsiloxane (PDMS).

With this fabrication process we successfully integrated CNT into the nanorobot. Figure 4 shows a picture and scanning electron microscope (SEM) images of fabricated nanorobot.

RESULTS AND DISCUSSION

To introduce the nanorobot into a workspace on a microfluidic chip, we utilized femtosecond laser ablation. By cutting holding pillar with femtosecond laser ablation, we utilized femtosecond laser ablation. By cutting holding pillar with femtosecond laser ablation, we can introduce the nanorobot into the workspace. Concept of the femtosecond laser ablation of the holding pillar is depicted in Figure 5. After cutting the holding pillar, nanorobot can be manipulated by HOT.

To evaluate optical manipulation characteristics of the nanorobot, we performed frequency response evaluation. Positions of the nanorobot were measured by a template matching based on images acquired by CCD camera attached on the microscope (Figure 6 (a)). We performed evaluations in three directions, considering symmetry of the nanorobot. Definitions of the direction is shown in Figure 6 (a). Figure 6 (b) and (c) show the gain diagram and phase diagram of the nanorobot when sinusoidal input with 2.0 μm amplitude were applied. We evaluated the response at the frequency 0.1 Hz, 0.5 Hz and every 1 Hz from 1 Hz to 10 Hz. Horizontal axis of Figure 6 (b) indicates the magnitude of amplitude of the nanorobot and Figure 6 (c) indicates the phase lag time of the nanorobot. These results indicated the nanorobot can follow the input up to a few Hz in all directions. This drive frequency is thought to be sufficient for cell application.
Furthermore, we have demonstrated a single cell puncture with the fabricated hybrid nanorobot. A cell puncture is one of the most important cell surgeries that is used for injection of DNA, siRNA or fluorescent probes into a cell. A cell puncture for single somatic cell has never been realized by previous on-chip robots. Therefore we performed single cell puncture by using photothermal characteristics of CNT [5].

The nanorobot was fixed on the glass substrate and target cell was transported to the CNT probe by using HOT. When the target cell was attached to the CNT probe, the IR laser was irradiated to the CNT and the target cell was punctured with a heat generated by the photothermal effect of the CNT. In this study, we used Madin-Darby canine kidney (MDCK) cell as targets and these were stained with fluorescent dye (Life Technologies Corporation, Cell Mask Plasma Membrane Stain) that can selectively stain cell membrane. Stained MDCK was dispersed in a culture medium and introduced into the fabricated chip. Additionally we introduced fluorescent nanobeads into solution in the chip. If the target cell was successfully punctured, nanobeads can be introduced into the target cell.

Figure 7 shows bright field (BF) image (Fig 7 (a)), fluorescent image of stained cell membrane (Fig. 7 (b)), and fluorescent image of nanobeads (Fig. 7 (c)) after laser irradiation. Fluorescent signal of nanobeads was detected inside of target cell in Figure 7 (c). Therefore, the target cell thought to be successfully punctured by the nanorobot and nanobeads was introduced.

Figure 7: Demonstration of single cell puncture: (a) Bright field image of target cell and nanorobot, (b) fluorescent image of stained cell membrane, (c) fluorescent image of nanobeads.

CONCLUSION

In this paper, we proposed on-chip nanorobot having nanometer size and hybrid structure with functional nanomaterials (hybrid nanorobot). This hybrid structure enables to create new function of the nanorobot by utilizing unique characteristics of the nanomaterials. We successfully fabricated proposed hybrid nanorobot with CNT and performed optical manipulation of the hybrid nanorobot by HOT. Furthermore, we demonstrated the single cell puncture by utilizing photothermal characteristic of CNT. We succeeded in puncture of the single cell with fixed hybrid nanorobot.

Proposed hybrid nanorobot enables manipulations and surgeries of small somatic cells. Moreover, by utilizing various characteristics of functional nanomaterials, novel functions can be created and integrated on on-chip robots.

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