Effect of Structure and Surface Treatment of CNTs on Micro High-Efficiency Bio-Analysis

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Abstract—**In this study, carbon nanotubes (CNTs) are anticipated as nano/micro structured reaction field for micro bio-analysis. CNTs reaction field were made on silicon substrate using chemical vapor deposition and self-organization of CNTs. To evaluate and reveal the optimal structure of CNTs structure, protein adsorption characteristics were evaluated with micro fluorometry. Furthermore, plasma ion irradiation was applied to treat the surface of CNT in order to improve the absorbability of proteins. The efficiency of surface treatment was evaluated by Raman spectrum analysis. By using fluorescence microscopy, it is revealed that the adsorbability of proteins depends on structure and surface treatment condition significantly and finer structures with proper treatment tend to adsorb more proteins.**

Index Terms—**Carbon nanotubes, self-organization, surface treatment, fluorometry.**

I. Introduction

Nano-materials are increasingly playing a key role in electronic, biological, chemical, and medical applications. In particular, carbon nanotubes (CNTs) have been attracting significant recent attention because of their unique characteristics [1]-[5]. Due to their unique properties, CNTs are being studied extensively [6], [7]. Based on their excellent electrical properties, such as high current densities exceeding 109 A/cm^2, CNTs are also being considered for application as nano-interconnects. If CNTs are bundled together at high density, they are anticipated to have a lower resistivity than Cu interconnects. Also, as next-generation semiconductor technologies, CNTs are being applied in electronic devices, such as field emission transistors (FETs). Due to their excellent mechanical properties, CNTs are finding applications in composite materials and as carbon fibers. We pay attention to using CNTs as a sensor while there are various studies such as the above. For instance, CNTs have been applied as electrodes in fuel cells, and CNT-based reactors have been used as reaction fields in biosensors. Because its chemical stability, nanostructure with high aspect ratio which brings in high specific surface area. Currently, CNTs can be obtained with lengths of about 1~100 μm, with corresponding aspect ratios of more than 1000.

The development of micro reactor for Lab-On-Chip (LOC) device, a term for "micro Total Analysis Systems (μTAS)", has been growing recent years [8], [9]. LOC devices are now widely used in biology and biotechnology, applications include analysis of DNA and proteins, high throughput screening, and chemical reactions. Various bio-substances are often analyzed by detecting the immune reaction between antigens in a sample solution or antibodies immobilized on micro channel-surfaces using fluorescence detection and the surface plasmon resonance method. In such applications, detection depends significantly on surface of reaction field. Also, reaction efficiency significantly depends on the surface's physical and chemical properties (such as specific surface area, wettability, adsorbability etc.). The design and creation of surfaces that have high specific surface area and arrows quicker analysis is currently one of the important issues in LOC and μTAS. One of such studies, CNTs is tried to be used as nano/micro structured reaction field [10], [11]. In those studies, reaction efficiency was enhanced by CNTs structure, but optimal structural parameter and surface characteristics of CNTs were not revealed.

In this study, to reveal the optimal structure and surface characteristics of CNTs, we propose to use open-well micro reactor with CNTs and protein adsorption characteristics were evaluated by fluorometry. Therefore the aims of this study were followings, 1) to fabricate micro reactor with CNTs which has several structural parameters easily, 2) to investigate the effect of surface treatment on the reaction efficiency. We made CNTs reaction field easily using Chemical Vapor Deposition (CVD) and clumping of CNTs by self-organization process. In this time, the wettability and crystallinity of CNT reaction field were controlled by Ar plasma irradiation. Reaction efficiency was evaluated with protein adhesion characteristics using fluorometry.

II. Experiments

A. Synthesis of CNTs

We synthesized CNTs by catalytic thermal chemical vapor deposition method because it allows us to synthesize aligned CNTs over large areas, and can be applied industrial [12], [13]. As substrates, we used silicon wafer with oxide film for fluorescence analysis. Fig. 1 shows the schematic view of the equipment used in this study (Microphase Co.). Evaporated ethanol was used as a source of carbon. Iron (Fe) and aluminum (Al) were sputter-deposited previously as catalysts for CVD by electron cyclotron resonance (ECR) type ion shower equipment (ELIONIX Co.) on the substrate. Rate of deposition of Al and Fe was 10~20 nm/min and 5~10 nm/min, respectively. Length of CNTs was controlled by changing thickness of Al and deposition time of Al and Fe were 1~5

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min and 1 min, respectively. CNTs synthesized by CVD method are very dense structure with high aspect ratio. Though, as a 3D-structured reaction field for microchip, it is too dense for reactants to spread over the surface and react. To achieve CNTs structure with enough space for reactant diffusion, the density of CNTs is needed to be controlled properly according to the size of the reactants.

B. Fabrication of CNTs Reaction Field

Synthesized CNTs were hydrophobic surface without any treatment. Contact between vertically aligned CNTs and a liquid is unavoidable when CNTs are used in a reaction field. However, the infiltration of a liquid is very difficult because the CNTs have a hydrophobic surface. Thus, establishing techniques for controlling the wettability of CNTs is important. To change CNTs wettability to hydrophilic, argon (Ar) plasma was irradiated [13]. CNTs exhibited super hydrophilicity after Ar treatment. ECR-type ion shower equipment is used again.

After Ar plasma irradiation, CNTs are clumped together and made bundles by self-organization, and we call these clumped and structured CNTs as "CNTs reaction field". Fig. 2 shows the process of making CNTs reaction field. Ethanol was dropped on the CNTs and dried in the atmosphere. By ethanol's osmosis and evaporation, CNTs are clumped together by meniscus force and keep its structure because of van der Waals' forces. We made various kinds of CNTs reaction field which have different density of CNTs structure. By changing length of CNTs, density of CNTs structure was changed at whole area of substrate. By encouraging ethanol's evaporation at the ventilated box, gaps of CNTs structure got various sizes in same area.

C. Surface Treatment Using Ion Plasma Irradiation

As mentioned above, if we use CNTs as reaction field, it is necessary to reform CNTs from hydrophobic to hydrophilic. Also, it is said that the protein tends to adsorb on the defects of reaction field. Thus, protein may become easy to be adsorbed on CNTs by introducing defects on tube structure. The authors had applied plasma irradiation to change the wettability of CNTs uniformly and efficiently in dry conditions, and to change the crystallinity as well [14]. The influence of the irradiation work volume, which is defined the duration time (min) at accelerating voltage (V), were evaluated on surface characteristics of CNTs by Raman spectrum analysis. In addition, a condition of ECR to use for catalyst deposition and surface treatment are shown in TABLE I.

D. Evaluation Method

Structure of CNTs and CNTs reaction field were observed by scanning electron microscope (KEYENCE Co.). The influence of irradiation conditions in wettability of CNTs was investigated. The wettability of the CNTs was evaluated by measuring the contact angle. Evaluation of the crystallinity was carried out using Raman spectroscopy. Typical Raman shifts (cm^{-1}) of CNTs include the G-band and the D-band. The G-band is a graphite-derived mode and its peak is at about 1590 cm-1. The D-band is disorder of the CNT-derived mode and its peak is at 1350 cm-1. The CNTs were characterized to obtain the ratio between the G-band and the D-band. Also, the RBM (Radial Breathing Mode) is an SWNT-derived mode. The CNTs are single-walled nano-tubes (SWNTs) if a peak of 248 cm^{-1} is observed. Therefore, if Raman spectrum peak was changed after Ar plasma irradiation, we could obtain CNTs which is suitable for adsorption of the protein.

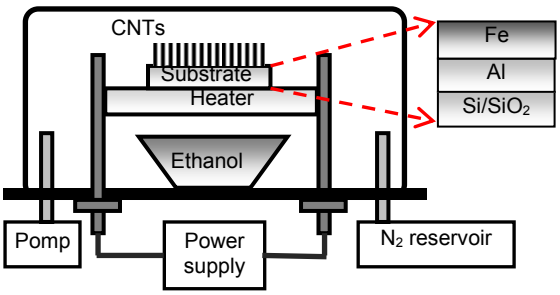


Fig. 1. Schematic image of CVD equipment and catalysts.

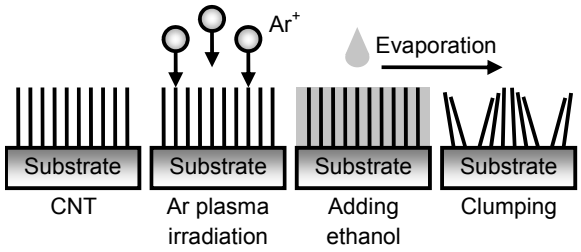


Fig. 2. Fabrication of CNTs reaction field.

To reveal the influences of structural parameters of CNTs structure for reaction and evaluate its reaction efficiency, Fluorometry was performed. We evaluated reaction efficiency by protein adsorption to the CNTs reaction field. At first, we revealed inclination of protein adsorption to the CNTs reaction field by fluorescence analysis with fluorescence labeling using antigen-antibody reaction. Fig. 3 shows schematic view of experiment and antigen-antibody reaction. First, 100 ml bovine serum albumin (BSA) solution (0.1 mg/ml) was dropped on the CNTs reaction field. Then, surface of CNTs structure was blocked by 100 ml skim milk. After that, 20 ml anti BSA with fluorescent isothiocyanate (FITC) solution was added to the sample. After each steps, the sample was incubated 30 minutes to fixing and reaction, and cleaned by 20 ml phosphate buffered saline (PBS) three times to remove superfluous after. The sample was observed by fluorescence microscope (Olympus Co.). Amount of adsorbed protein was evaluated by observing fluorescent intensity of the sample. To reveal inclination of protein adhesion to various CNTs structure, we used CNTs reaction field which has various gaps of CNTs structure. About one condition, we prepared three substrates and we measured three points each. After that, we assumed the average as fluorescence intensity.

TABLE I: ECR CONDITION

	Accelerating voltage [V]	Duration time [min]
Iron deposition	**1250**	**1min**
Aluminum deposition	**1250**	**1, 3, 5**
Wettability transform	**500**	**1, 2, 3, 4, 5**
Crystallinity transform	**1000**	**10, 20, 30, 60**

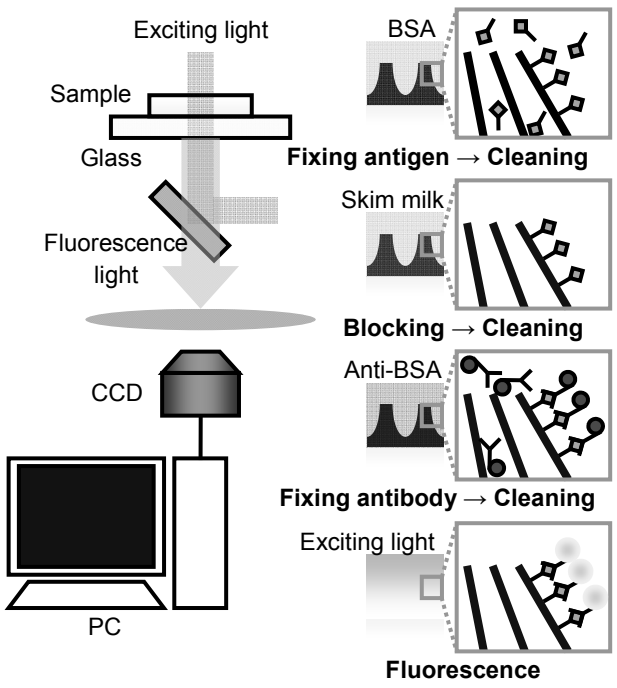


Fig. 3. Schematic image of fluorescence and antigen-antibody reaction.

III. Results and Discussions

A. CNTs Reaction Field

Fig. 4 shows SEM images of CNTs and CNTs reaction field synthesized and fabricated in this study. Fig. 4 (b) (d) (f) CNTs reaction fields were fabricated by clumping each Fig. 4 (a) (c) (e) CNTs respectively. CNTs grew up dense and vertically aligned on the substrate by CVD. Length of CNTs were 2~20 μm by changing condition of catalysts as mentioned previously. CNTs are clumped up and made bundles. Width of gaps are approximately 1~10 μm at the top of bundles, approximately 1~30 μm at the bottom of bundles and they got wider as CNTs got longer. In addition, the error bar of the gap of the bundles that we can get grows bigger so that CNTs length is longer. Therefore, we succeeded in manufacture of the reaction fields to have various microstructure in the CNTs of the nanostructure body. It is used to observe protein adsorption to CNTs structures which have different gaps in a chip, and to make finer CNTs structure.

B. Effect of Ion Irradiation on CNTs Surface Structure

Fig. 5 shows photo images of the contact angles for vertically aligned CNT film and the change in the contact angle of CNTs for different irradiation conditions. All the droplets were deionized water with a volume of 1 μl. The results show that the contact angle for the as-synthesized vertically aligned CNT film was 145 degrees, and that for the film irradiated at 500 V was close to 0 degrees. The experimental results show that the wettability of the CNTs can be modified by the ion irradiation, and the hydrophilic character of the CNTs was appeared with voltages of 500 V more significantly than that with voltages of 100 V. This means that modification does not occur unless the plasma ions exceed a specific energy threshold value. Since the contact angle for a drop of water on graphite sheet is around 85 degrees, it is thought that the hydrophobicity of the vertically aligned CNTs is not due to its chemical properties, but due to the tube structure and surface bundles. Its hydrophobicity is likely not stable.

Fig. 6 shows the results of Raman spectroscopic analysis of CNTs for different irradiation conditions. In the red plot the peak of G band and the D band was clear before plasma processing and understands that formed CNTs is the graphite sheet that there are slightly many defects. In addition, our synthesized CNTs were multi walled CNTs because RBM mode could not be observed.

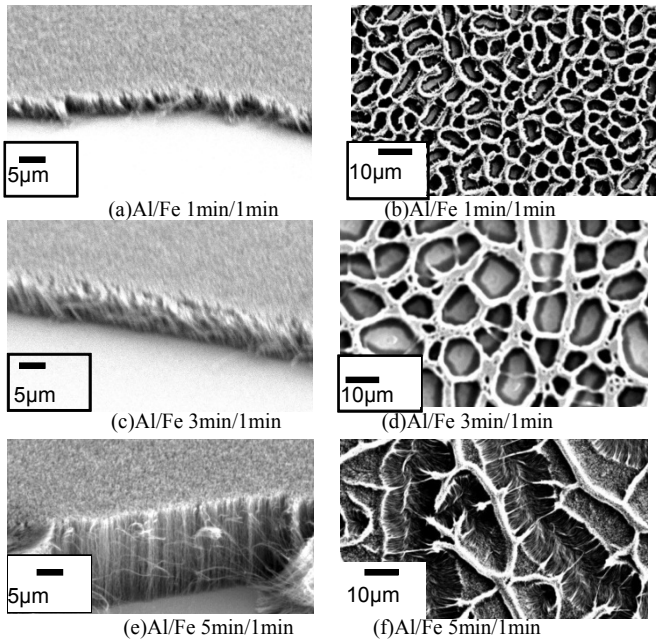


Fig. 4. SEM image of (a) (c) (e) CNTs and (b) (d) (f) CNTs reaction field.

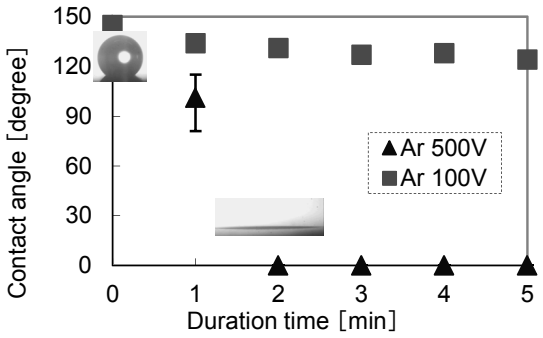


Fig. 5. Contact angle of the vertically aligned CNTs film which irradiated with various Ar plasma conditions.

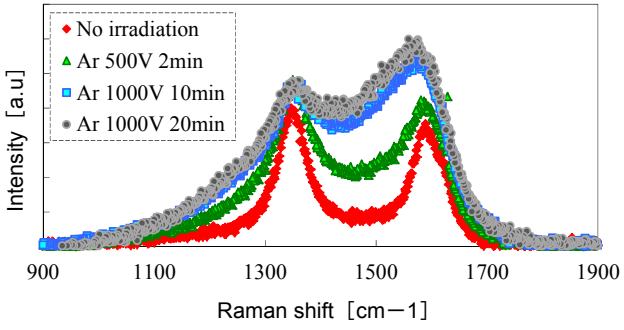


Fig. 6. Raman spectrum change by Ar plasma irradiation.

In the case of irradiation with small irradiation work

volume for controlling wettability, for example at 500 V for 2 min, there was little change in the Raman spectrum. This resembles the change in the Raman spectrum change for modifying the wettability by the introduction of functional groups.

In contrast, the Raman spectrum change was much larger in the case of large irradiation work volume, for example at 1000 V for 10 min. This indicates that destruction of the outer CNTs grapheme sheets structure [14]. The crystal structure were different from graphite because the spectra could be fitted to an amorphous pattern [14]. In addition, we used CNTs within less than 10 minutes duration time for reaction field, because the removal of CNTs occurred after more than 20 minutes duration time.

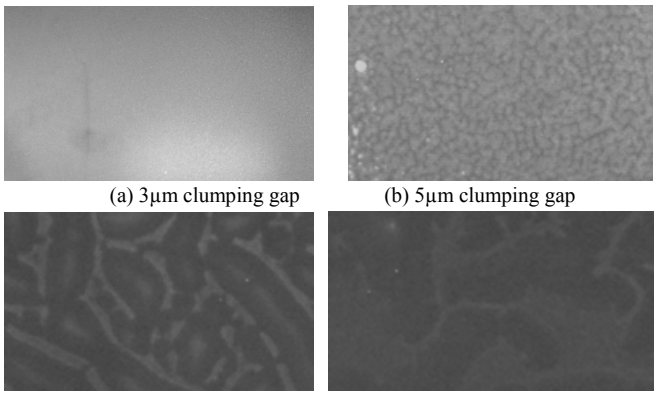
C. Protein Adsorption Characteristics

Fig. 7 shows fluorescence image of CNTs reaction fields taken by CCD. BSA were labeled with FITC, so brighter areas had been adsorbed more BSA than darker areas. In this time, CNTs in Fig. 7 (a)~(d) has each clumping gap and plasma processing was only wettability transforming. Control of the clumping gap is just what we mentioned it earlier. From Fig. 7, it can be said that BSA tend to adsorb to top of CNTs more than side (side of groove structure), especially to edges of groove structure which has width of ~10 μm. BSA adsorbed to side of CNTs structure though it looked dark. Amount of adsorbed BSA were bigger when the size of CNTs structure got smaller. The fluorescent intensities for each CNTs reaction field which has several clumping gap are summarized in Fig.8. Intensities got about 2 times bigger when gaps width got 1/10 smaller. Also, at top of CNTs structure, edge of CNTs structure got 1.5~ 2.0 times bigger than other part, and at side of CNTs structure, fluorescent.

From the results of fluorescence analysis, it can be explained like following Biological materials (like protein and cell) tend to adsorb to tips of micro structure. From that, BSA adsorbed to tip of CNTs structure much more than side. Furthermore, in this study, top of CNTs structure has nano metal particles, on it, and been affected by Ar plasma irradiation. Those conditions influenced the protein adsorption and fixing to CNTs reaction field. About adsorption to the side of CNTs structure, it can be explained like following. After adding BSA solution, BSA diffused approached and adsorbed to CNTs structure. When the widths of CNTs structure gaps got 1/10, it meant decreasing of distance for diffusion and approaching, and adsorption. As a result, protein got adsorbed to CNTs structure much more than wider CNTs structure gaps. From these, it can be said that protein adsorption can be greatly encouraged by using CNTs reaction field which has less 5 μm gaps of CNTs structure. It is not true that the width of gaps have to be smaller, because it is hard for solution and reactant to get through and spread.

Fig. 9 shows fluorescence image with various CNTs surface characteristics which has approximately 10 μm clumping gap. Fig.9 (a) was for original CNTs without the plasma processing and Fig.9 (b) for wettability transformed CNTs. Fig. 9 (c) and (d) were the images after wettability transforming for plasma processing with higher energy degrees, respectively.

Amount of adsorption of BSA were bigger when the Ar plasma irradiation to CNTs got larger. It can be said that BSA tend to adsorb on defects on the surface of CNTs more. Protein becomes easy to adsorb on CNTs more when we treated CNTs surface using the plasma processing with higher energy degree to introduce more defects on the surface of CNTs, which Raman spectrum changes more than other conditions as shown by the blue square dots in Fig. 6.



(a) 3μm clumping gap (b) 5μm clumping gap

(c) 10μm clumping gap (d) 20μm clumping gap

Fig. 7. Fluorescence images with CNTs reaction field which has several clumping gap.

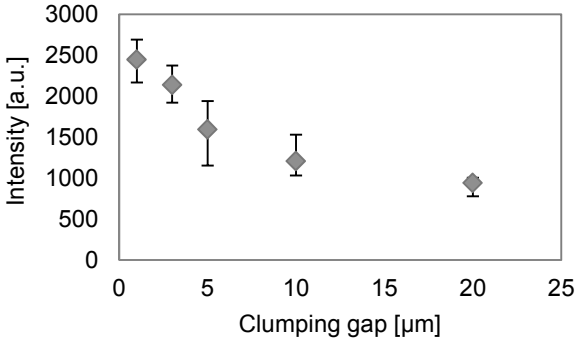
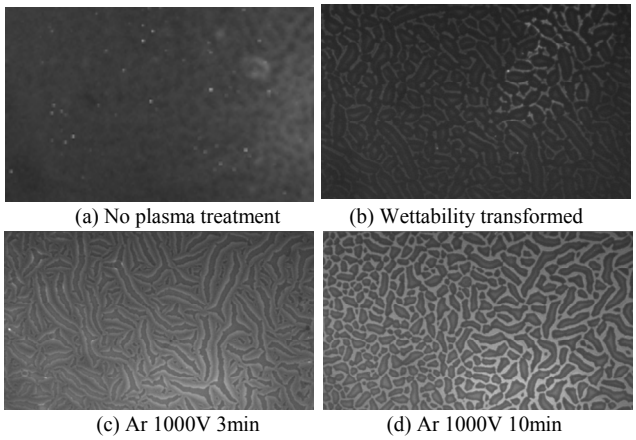


Fig. 8. Relationship between CNTs clumping gap and fluorescence intensity.



(a) No plasma treatment (b) Wettability transformed

(c) Ar 1000V 3min (d) Ar 1000V 10min

Fig. 9. Fluorescence images with CNTs reaction field which has several surface characteristics.

The fluorescent intensities for each CNTs reaction field which has several surface characteristics are summarized in Fig.10. We prepared the substrate which conducted an antibody antigen reaction in Si without CNTs as a comparison here. Intensities got about 2 times bigger when Ar plasma processing got larger. It is thought that protein becomes easy to adsorb on CNTs because a graphite sheet is

broken and crystal characteristics are near amorphous [14]. This accords with the previous study that made it easy to add protein to CNT sidewall using a chemical solution [15].

For example, the adsorption equations of Langmuir expressed the relationship adsorption of adsorptive medium with adsorbed molecule. There is an adsorption site of limited number in adsorptive medium and is combined with adsorbed molecules only there. Also, all adsorption sites are equivalent, which mean that one adsorption site is combined with only one adsorbed molecule. Therefore, it is thought that the plasma processing to CNTs increases the numbers of a limited adsorption site by the defects introduction to CNTs surface.

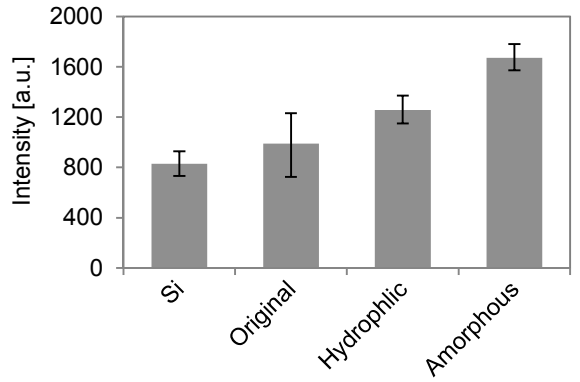


Fig. 10. Relationship between CNTs surface characteristics and fluorescence intensity.

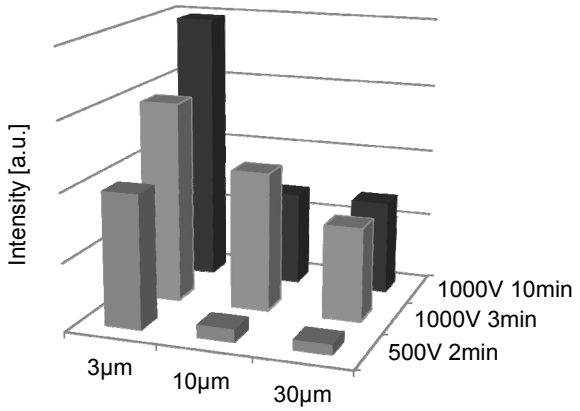


Fig. 11. Fluorescence intensity when we combined plasma processing with structure control.

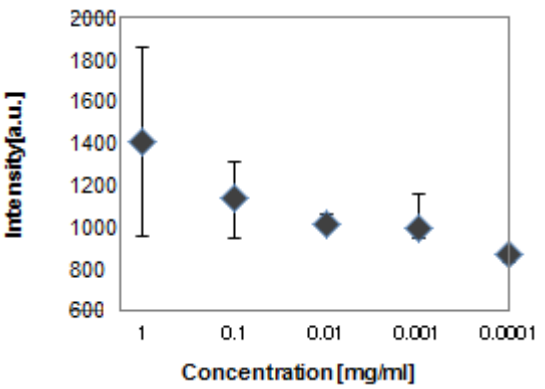


Fig. 12. Fluorescence intensity with changing concentration of BSA solution.

From now, we mention fluorescence intensity when we combined plasma processing with the control of structure dimensions by clumping in Fig. 11. In this time, fluorescence intensity remove a background noise. In other words, we remove the fluorescence intensity of the substrate which we block it and added only anti-BSA.

At first, as mentioned above, fluorescence intensities increase when clumping gap shortens or quantity of the plasma processing increases. However, the fluorescence intensity was saturated when plasma processing increases to a certain quantity unless a clumping gap was short. It is thought that this is because structure itself changes not defects introduction to CNT surface when we exceed certain quantity of plasma processing. In addition, fluorescence intensity with much plasma irradiation was smaller than the substrate which clumping gap is small even if I handle a lot of plasma. The adsorption sites increased by plasma processing, but it may be a reason that there was little protein which hits the adsorption site because clumping gap was big. Especially, Ar plasma irradiation which has bigger Raman spectrum change as shown in figure 6 was very effective for improvement of the reaction efficiency. In combining plasma processing with the finer reaction field fabrication, fluorescence intensity of up to 30 times could be obtained.

From results above, we evaluated CNTs reaction field which has amorphous surface and 3 μm clumping gap at various concentration of BSA solution. If we apply CNTs as the bio-sensor, the linear nature and detection limit for the quantity of adsorption of the protein are important. Fig, 12 shows fluorescence intensity with changing concentration of BSA solution. Fluorescence intensity got decreased when BSA solution got less concentrated. In consideration of the background noise (In this time, approximately 700), enough intensities of fluorescence were provided by all concentration. In addition, when density was high, an error bar was big, but was able to confirm the linear nature generally. In this case, 1.0 mg/ml and 0.1 μg/ml mean 17 μmol/l and 1.7 nmol/l, the detection limit with the process in this study is considered at around 1 nmol/l.

IV. Conclusion

In this study, we fabricated nano/micro reaction field fro bio-analysis using CNTs and evaluated its reaction efficiency by fluorescence analysis. The results are following. (1) Without plasma processing, protein adsorbed to tip of CNTs structure rather than side of CNTs structure. (2) Reaction efficiency of CNTs reaction field as nano/micro structured reaction field can be enhanced by making its structure dense like a few μm of gaps between each structure. (3) Ar plasma irradiation to CNTs can be used for wettability and crystallinity transforming. (4) In combining plasma processing with careful reaction field fabrication, fluorescence intensity of up to 30 times could be obtained. (5) In fluorescence analysis, detection limit in this study was about 1 nmol/l.

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