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Selective Biomolecules Immobilization onto Plasma-Functionalized Carbon Nanotube Dot Array using Capillary Atmospheric Pressure Plasma Jet for Microarray Biosensor

キャピラリー大気圧プラズマジェットによりプラズマ修飾したカーボンナノチューブアレイ上への選択的バイオ分子の固定化

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To immobilize biomolecules onto carbon nanotubes (CNTs) dot array, a capillary atmospheric pressure plasma jet (APPJ) technique is developed. An APPJ with a micro-capillary was used to functionalize amino groups to obtain plasma-functionalized CNTs dot array. After then, to immobilize biomolecules selectively we employed biotin-avidin system in place of antibody and antigen reaction. The successful functionalization using APPJ and simulating biotin-avidin system onto CNTs dot array are valuable for future application of microarray biosensor.

1. Introduction

Carbon nanotubes (CNTs) have many great interests for sensitive detection of biomolecules [1]. Successful realization of biosensors based on CNTs requires proper control of their functionalization and surface immobilization. Compared to the conventional chemical functionalization treatments, plasma treatments have the advantages, such as low temperature and little damaging effects [2]. Among the plasma treatments, atmospheric pressure plasma treatment has attracted many researchers. In this work, a capillary atmospheric pressure plasma jet (APPJ) technique is developed to functionalize amino groups on CNTs dot array selectively. The feasibility of the techniques for microarray biosensor was demonstrated by successfully functionalize CNTs dot array using capillary APPJ and simulate biotin-avidin immobilization selectively onto CNTs dot array.

2. Experimental

The fabrication of CNTs dot array was performed by electron beam lithography and a combined thermal and plasma-enhanced CVD method. FE-SEM image of CNTs dot array is shown in Fig.1. The fabrication method of vertical aligned CNTs dot array has been reported in detail elsewhere [3].

For the surface functionalizaition of CNTs dot array, an APPJ with a micro-capillary was used to functionalize amino groups. Schematic of the APPJ experimental setup is shown in Fig.1. Plasma discharge was produced in the glass tube under the high voltage square-wave pulses of ±7.5 kV at a frequency of 5 kHz and 50 % duty ratio.

Moreover, to immobilize biomolecules selectively we employed biotin-avidin system in place of antibody and antigen reaction. Poly(ethylene glycol), PEG, was introduced for resisting nonspecific binding of biomolecules on CNTs dot array [4]. The success of the selective biomolecules immobilization was confirmed by chemical derivatization with the fluorescent dye which can be visualized by fluorescent microscope.

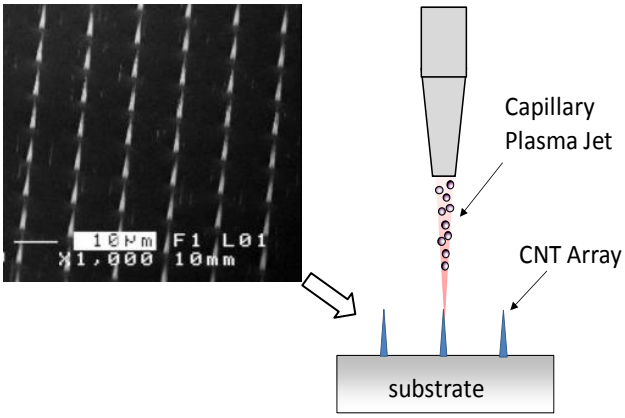

Fig.1. FE-SEM image of CNTs dot array and schematic of surface treatment by APPJ.

3. Results and Discussion

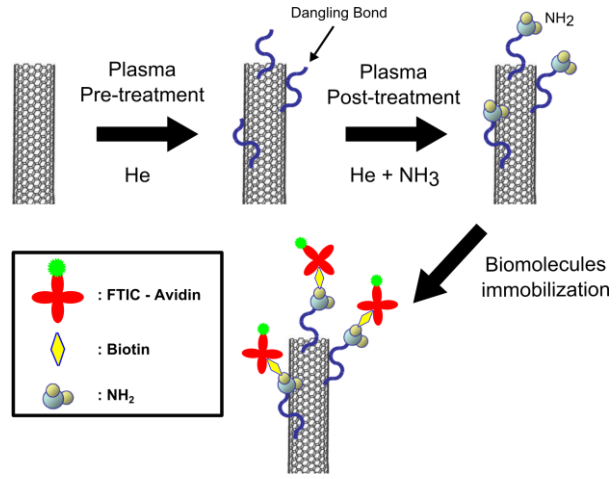

Fig.2. Proposed mechanism of the surface immobilization of CNTs.

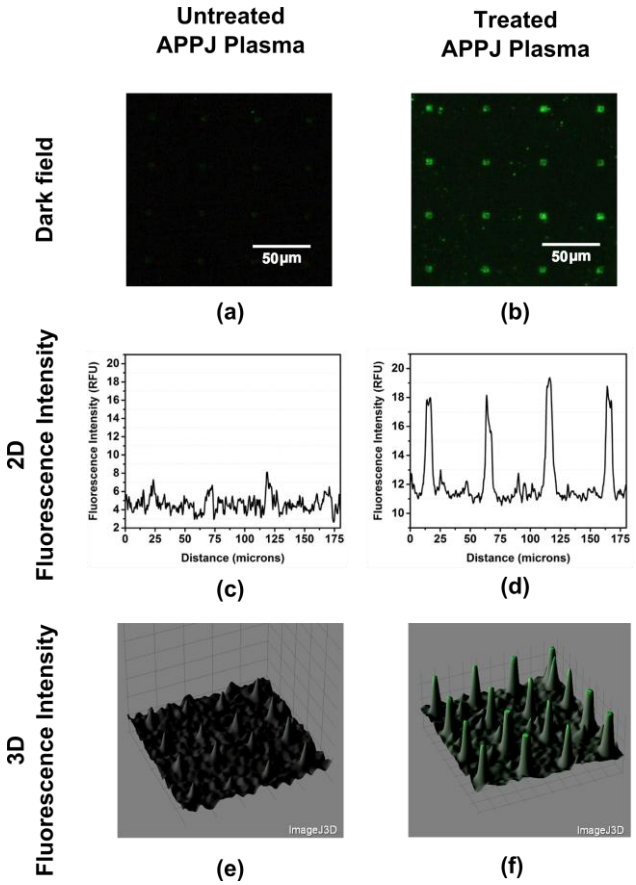

Fig.3. Comparison between untreated and treated APPJ plasma obtained from CNTs dot array performed biomolecules immobilization. (a) and (b): dark field image covered with Avidin-Biotin; (c) and (d): 2D fluorescence intensity from Figs. 3a and 3b; (e) and (f): 3D fluorescence intensity from Figs. 3a and 3b.

The proposed mechanism of the surface functionalization and biomolecules immobilization of CNTs is shown in Fig.2. The surface functionalization was carried out by applied two-stage plasma treatment: (1) pre-treatment by using He discharge gas with -500 V dc biasing and (2) post-treatment by using a mixture of helium and ammonia without bias. In the pre-treatment, ion bombardment was the dominant process to activate CNTs by creating Dangling bonds [5]. The Dangling bonds were created by ion bobmardment during pre-treatment process to ease functional group introduction onto CNTs in the post-treatment. The biomolecules immobilization was simulated by PEG-biotin-avidin-fluorescein isothiocyanate (FTIC) immobilization. Fig.3 shows comparison between untreated and treated APPJ plasma obtained from CNTs dot array of 4×50 μm spots performed biomolecules immobilization. The green areas in Fig.3b correspond to fluorescent dye of avidin (FTIC-avidin) connected to biotin and amino functionalized CNT dot array. The black areas in Fig.3a indicate that fluorescent dye of FTIC-avidin is not connected to amino functionalized CNT dot array. Good spot uniformity of biomolecules immobilization are confirmed by 2D and 3D fluorescence intensity line profile across CNT dot-array of 4x50 μm showed in Figs.3d and 3f.

4. Conclusion

We studied the immobilization of biomolecules onto CNTs dot array using a capillary APPJ technique and demonstrated to functionalize the CNTs dot array. To immobilize biomolecules selectively, we employed biotin- avidin system in place of antibody and antigen reaction. The successful functionalization using APPJ and simulating biotin-avidin system onto CNTs dot array are valuable for future application of microarray biosensor.

Acknowledgements

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