ABSTRACT

Due to the exceptional characteristics of carbon nanotubes (CNT), extensive research have been given to carbon nanotubes and its application to analytical science. The study demonstrates the ability of an array of multiwall carbon nanotubes (MWCNT) to absorbed proteins from its bulk solution by leveraging on its super hydrophobicity and the absorption properties of MWCNT. The absorption is shown to be motivated by primarily the concentration effect and the absorptivity of the proteins.

INTRODUCTION

Carbon nanotubes(CNT) is one of the key driving forces in the area of research of nanotechnology for its unique structural, mechanical and chemical properties. While CNTs can be synthesized by techniques such as laser ablation, catalytic arc discharged and chemical vapor deposition(Baughman, 2004 ), chemical vapor deposition was chosen due to its unique advantage of synthesizing of vertically aligned carbon nanotubes. CNT is expected to have high biocompatibility due to the ability of π electrons interact with organic molecules and biomolecules(Valcarcel, 2005). Hence, a MWCNT array can be used directly in the design of a biosensor making use of the MWCNT array for protein immobilization.

Another unique characteristic of nanostructures is its ability to form superhydrophobic surfaces most commonly seen in morning dew rolling of a lotus leaf(Xi, 2009 ). The superhydrophicity occurs in these nanostructures because the inherent roughness of the nanostructured CNT. Coupled with strength of support from the CNT array, the droplet is essentially supported on a layer of air. This work explores the ability of such stable superhydrophobic surfaces for analytical purposes.

An experiment is designed for the separation of proteins from its bulk solution through the diffusion of proteins from a superhydrophobic MWCNT array is designed. This is done by a simple and direct application of protein droplets onto an array of dry MWCNT so that its superhydrophobicity is maintained while allowing the proteins to be absorbed into the MWCNT array taking advantage of bio-nano adsorption of proteins by CNT through conjugation of hydrophopic pockets of proteins and hydrophobic surfaces of CNTs(Zhong, 2009).
EXPERIMENTAL

Synthesis of CNT
An array of MWCNT has been grown on a silicon substrate. The MWCNT array was grown by a 2 step process by coating a thin film of iron catalyst by radio frequency assisted sputtering followed by MWCNT growth by plasma enhanced chemical vapor deposition (PECVD).

Coating of Fe catalyst
The sputtering of iron on 10mm by 5mm substrate was done for 6 minutes with a power of 100W of the radio frequency (RF) source under high vacuum with rotation of the stage.

CNT growing
The CNT was grown by PECVD method using PlasmaQuest Series III, PQM-9157-A. The iron coated substrate was slowly heated to 610°C. After which, the plasma was first formed with hydrogen gas at a flow rate of 60 sccm. The MWCNT growth was initiated with the introduction of the carbon source, acetylene gas, at a flow rate of 15.5 sccm and flow rate was maintained for 1 hour. The plasma will function as an energy source to decompose the acetylene to release reactive carbon atoms which will diffuse to the substrate. The heated layer of iron would then act as a catalyst for the growth of CNT.

Preparation of Alexa Fluor 430 conjugated protein
5 proteins (Hemoglobin, Myoglobin, Lysozyme, Albumin from Bovine Serum (BSA) and Albumin from chicken egg white) were conjugated with Alexa Fluor 430 obtained from Invitrogen in a sodium bicarbonate solution.

Separation of Alexa Fluor 430 conjugated protein and the unreacted dye
The conjugated protein is purified with gel permeation chromatography using NAP-10 columns containing Sephadex G-25 from GE Healthcare to obtain a 10mg/ml of protein conjugated with Alexa Fluor 430.

Application of protein onto CNT
5.0μL of the conjugated proteins was dropped onto the CNT surface using a micropipette without touching the MWCNT array. The droplet was also applied as gently as possible to prevent the droplet from bouncing of the hydrophilic MWCNT array.

Development of the protein spreading
2 environments were used to develop the spreading protein on the MWCNT array. Firstly, the protein droplet was allowed to spread in a water tank over 2 weeks so that the droplet was given enough time to reach equilibrium without evaporation. Another environment was allowing the droplet to spread in a 3cm wide sealed glass petri dish over 4 hours allowing the droplet to evaporate to dryness.

Epifluorescence Microscopy
Epifluorescence microscope was used to capture the image of the spreading of fluorescent dye conjugated protein. The green light emitted by the excitation using a blue light source of the dye-conjugated protein is then observed under the Epifluorescence Optika Microscope and captured using the sensovation camera SamBa EZ-130c.
**Scanning Electron Microscopy**

The samples were placed on the stage using double-sided carbon tape and sputtered with Platinum using JEOL 1600 at 6Pa and a sputtering current of 20mA for 30 seconds. The samples are then loaded into the SEM chamber and the SEM micrographs were obtained.

**RESULTS AND DISCUSSION**

**Verification of proposed spreading mechanism by SEM and protein fluorescence microscopy**

**Proposed Mechanism**

1. Droplet lands with a superhydrophobic effect maintaining a round shape.
2. The MWCNT array absorbs the protein while repelling the solvent.
3. The process continues until all protein is absorbed hence giving a final contact angle and hence a fixed area of absorbance when the solvent is dried.

**Formation of microchannel**

There is horizontal stretching of CNT at the ending of the interface as it is evident under SEM. At this interface of movement, the proteins have a high concentration. The proteins then push the droplet radially outwards to achieve an even concentration gradient which is shown below.
Effect of concentration of protein

From left: 2, 1, 0.5, 0.2, 0.1mg/ml of Alexa Fluor Conjugated BSA absorption

It is shown that the presence of the amount protein directly influence the absorption on MWCNT array for both BSA and hemoglobin. The concentration dependence of the absorption also further demonstrates the selective absorption of proteins from its bulk solution.

CONCLUSION

The ability of a superhydrophobic surface of MWCNT to absorb proteins is shown in this work and the mechanism for absorption is established by analysis of SEM and epifluorescence microscopy. The absorption of proteins by the MWCNT array is also demonstrated to be motivated by the concentration and the absorptivity of proteins as shown. The proteins were also easily removed from its bulk solution into the MWCNT array.

In conclusion, the unique hydrophobic and absorption properties of this MWCNT array are leverage on to achieve the aim of using a MWCNT array for qualitative and quantitative biosensing of proteins in this work.

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