Self-Assembly Carbon Nanotubes on Cantilever Biosensor for Sensitivity Enhancement

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Abstract. In recent years, highly sensitive and selective as well as cost-effective sensing and detection of biomolecules (e.g. virus, bacterial, DNA and protein) by MEMS/NEMS (Micro-/Nano Electro-Mechanical-System) structures have attracted extensive attention for its importance in clinical diagnostics, treatment, and various genome projects. Meanwhile, substantial research efforts have been spent on the improvement of sensitivity of bioMEMS structures. Among a variety of methods that have been investigated, surface modification by nanoparticles (NPs) turns out to be an attractive way, which provides a platform for the enhancement of the sensitivity for biosensor devices. However, conventional applications for surface modification were mostly implemented on microelectrodes. Thus, in this paper, we demonstrate a new approach for surface enhancement on Au-coated silicon microcantilevers in micro-/nano-system. By self-assembly surface binding of multi-walled carbon nanotubes (MWCNTs) on the Au monolayer on top of the Si microcantilever surfaces, much larger surface area could be created for bio-molecular binding (such as antibodies or single DNA strands, which act as probes to capture target molecules). Therefore, this could enable specific interactions and selective binding to target biomolecules with a very low sample size, which greatly increases the sensitivity of detection. It should be noted that functionalization of MWCNTs with terminal carboxylic functionalities (in DCC solution) onto the Au surfaces of Si microchips have been introduced in our study. Further applications of MWCNTs functionalization are worth exploring in biomolecular detection for their exceptional mechanical and unique electronic properties. The successful binding of MWCNTs was testified as shown obviously on AFM image (Figure 1 and 2).

1. Introduction

Recently, sensing of biomolecules (e.g. virus, bacterial, DNA and protein) by MEMS/NEMS (Micro-/Nano Electro-Mechanical-System) structures (such as micro/nano-cantilevers) have attracted extensive attention for its importance in clinical diagnostics, treatment, and various genome projects. The analyte-receptor binding can be detected either by cantilever deflection in static mode due to surface stress or resonance method by measuring the resonant frequency shift in dynamic mode of the cantilever [1]. Meanwhile, substantial research efforts have been spent on the improvement of sensitivity of bioMEMS structures. As we know, probe molecules such as antibodies and ssDNA are
directly bound to the MEMS/NEMS structure surface previously to capture the targeted molecules through molecular interactions. Whereas, with the help of self-assembly surface binding of special materials, much larger surface area of the cantilevers could be created for bio-molecular binding. In other words, larger number of probe molecules on the surfaces could interact with targeted molecule under certain concentration. Therefore, larger resonance frequency shift or larger static deflection can be detected and more clearly observed when the sensor is exposed to certain amount of targeted molecules due to the increase in binding. Serving as an amplifier to improve detection signals, surface enhancement enables specific interactions and selective binding to target biomolecules with a very low sample size, which greatly increases the sensitivity of detection.

Among a variety of methods and materials that have been investigated, metal nanoparticles offer a unique set of physical properties that may be exploited in biological detection assays [2]. Surface modification by Au nanoparticles (NPs) turns out to be an attractive way, which provides a platform for the enhancement of the sensitivity for biosensor devices [2-4]. As an alternative material of NPs, carbon nanotubes, a seamlessly rolled up graphene sheets of carbon, have already showed their unique mechanical and electronic in their potential bio-sensing applications [5]. Further studies on functionalization of CNTs by biomolecules such as DNA or bovine serum albumin (BSA) have been intensified [3, 5-8].

However, conventional applications for surface modification were mostly carried out on microelectrodes with smaller molecules to be further attached. Au NPs or SWCNTs were mostly chosen to be experimented in most studies [4, 9-11]. In this project, in order to develop a flexible and portable biosensor which achieves high sensitivity with satisfied reproducibility as well as cost-effectiveness, we introduced a novel surface sensitivity enhancement method. More cost-effective MWCNTs were examined and functionalized to enlarge the attachment capacity of probes on Au monolayer of SiN microcantilevers.

Due to the limited time and resources only the surface functionalization of MWCNTs/Au NPs on Au-coated SiN microcantilevers was demonstrated, which is later determined by the resonance frequency shift detection after modification. This combination provides convenience, low-cost, desired reproducibility as well as high sensitivity. The LPCVD SiN low-stress rectangular cantilever (600µm long, 2.35µm thick, fabricated) as well as triangular microcantilevers (commercially available, Nanoprobe™) were both adopted in our experiments (Fig.1). Quality factor (Q factor) of the damping of the vibrating microcantilever, was calculated as the ratio of resonant frequency over its bandwidth (the full width at half maximum) and compared based on those ‘Amplitude Vs Frequency’ graphs constructed from the experimental data collected.

2. Experimental Details
As we can see, the change in resonant frequency as a function of the mass binding on the microcantilever surfaces forms the basis of the detection scheme.

2.1. Surface cleaning of the SiN microcantilever
Two reagents were utilized in this critical surface cleaning process to determine whether surface cleaning had any effect on resonance frequency. The 1st sample was immersed in newly-prepared piranah solution at around 70°C for 15mins. The cantilever was then taken out to be washed with DI
H₂O and dried in air. The other triangular cantilever was rinsed by IPA, followed by acetone and DI H₂O for 3 times also. Resonant frequency values were recorded after each washing accordingly.

2.2. MWCNT-COOH functionalization of Au-coated SiN microcantilevers

The chemical pathway we were using to chemically bind the MWCNTs (300nm-500nm, Sigma) to the Au surface through thiol-links is described as follows. Firstly, preparation of shortened MWCNTs with terminal carboxylic functionalities was essential. 10 mg of the raw MWCNTs were poured and suspended in the 20ml of a 3:1 acid mixture of concentrated H₂SO₄ (98%) and HNO₃ (65%). The test tube containing the MWCNTs and acids are then placed inside a beaker and ultrasonicated in a water bath for not less than 8 hours at ca. 50°C. After that, the mixture was diluted and centrifuged at 14000rpm for 5mins until the upper colorless liquid was decanted whereas the resulting black precipitate (acid-treated MWCNTs) was left at the bottom of the tube. The upper liquid was pipetted out and the acid-treated MWCNTs at the bottom were washed with DI water till pH was around 7. The intermediate step was to introduce cysteamine on Au surface of the cantilevers to be further linked to MWCNT-COOH. The Au-coated SiN cantilevers were immersed in 10mM of cysteamine/ethanol solution overnight at 4°C for self-assembly of the S-NH₂-. The final stage was carried out as follows: 10mM of dicyclohexylcarbodiimide (DCC) in the presence of N, N-dimethylformamide (DMF) was prepared firstly. The cysteamine-modified SiN cantilever samples were then soaked inside 5g/L shortened MWCNTs in the DCC and DMF solution. After 12hours, the sample were rinsed with ethanol and dried under optic illuminator. In this way, the shortened MWCNTs with terminal carboxylic acid functionalities are covalently linked to the amino groups of the cysteamine monolayer-functionalized Au surfaces of the cantilevers for later functionalization.

3. Results and Discussion

3.1. Reliability test for resonant frequency of the triangular SiN cantilevers

After surface cleaning process, the three average resonant frequency values along with the corresponding standard deviations after each washing were compiled into one graph for easier comparison. Apparently, as shown in Figure 2, the resonance frequencies before and after washing turned out to be quite consistent. No significant shift was detected and thus these results should be reliable for further usage. The maximum variations of resonance frequency among the 3 results did not exceed 0.17% for piranah washing and 0.99% in the case of regular rinse, which implied a good reproducibility of the resonance frequency measurement in dynamic mode. However, the quality factor was poor for both cases, which is not recommended for sensing purpose.
3.2. Surface appearance and characteristics after MWCNT functionalization on Au-coated SiN cantilever

Fig. 3 (a) is the SEM image of the 60° side view of the SiN triangular microcantilever. Fig. 3(b) shows the detailed view of the amplified surface area within the blue rectangular (in Fig.3(a)), where some several-micro-long structures of 270~280nm in width were found. It is reasonable to infer that the MWCNTs had been successfully bound to the surfaces of triangular microcantilevers. Those structures which were probably MWCNTs can be also clearly observed from Fig. 3(c).

Moreover, Figure 4 is an AFM image that reveals the appearance of Au-surface of SiN cantilevers before and after treatment of CNTs. The alignment of MWCNTs was not vertically standing upright on the surface as reported, but lying flatly close to the surface. Accumulation of several MWCNTs did occur as the diameter and length observed on the image was much larger than that of a single CNT, which reached as much as 20um long or 2um wide. Intersection of several CNTs could be seen obviously. These configurations might have drawbacks on the further binding of probe molecules such as DNA and antibodies, as COOH- functionalities were mostly at the ends rather than the sidewalls of the MWCNTs to attract probe molecules; whereas due to the horizontal alignment of MWCNTs on the cantilever surfaces, probes may be less likely to be bound to the ends. Therefore, the MWCNT functionalization process need to be further refined. However, a clear resonance frequency shift of 1.2 kHz was displayed on the signal analyzer with a high reproducibility and Q factor. Resonance was at 53250 Hz before and 52050Hz was that after MWCNT binding (Figure 5). The MWCNTs mass binding was estimated to be as much as 1.74±1.7ng applying formula by Gerber [13]. The absolute shift turned out to be quite large with small mass binding since the resonant frequency was extremely large for this specifically small triangular SiN cantilever.
Figure 3 SEM images of MWCNTs which was functionalized on surface of the small triangular cantilever

Figure 4 AFM images of surface of triangular microcantilevers before (a) and after (b) MWCNTs functionalization

Figure 5 Resonance frequency of the small V-shaped triangular SiN microcantilever before MWCNT functionalization (blue) and after MWCNTs binding (purple)

4. Conclusion
This paper introduced the basic idea of surface sensitivity enhancement of cantilever-based biosensor for biomolecular detections. Detailed descriptions of surface modification of MWCNTs procedures were provided. It is concluded that the detection of mass binding by resonant frequency shift method should be feasible for biosensing, with satisfied reproducibility and reliability after chemical washing, e.g. piranah and IPA. As probe molecules are immobilized on the sensor surface through MWCNTs, further shift of resonance frequency is expected to be detectable. With the help of CNTs, sensitivity could be considerably improved after surface binding area improvement. There are several limitations during the experimentation. For instance, error might occur during data manipulation or due to surface contamination. Also, the unexpected alignments of MWCNTs after functionalization on cantilever surface requires refinement such as cutting the MWCNTs even shorter or adding COO- to reduce the binding degree in order to avoid accumulation of CNTs. Moreover, the reproducibility will probably become a problem when entering the bio-detection stage, which requires further in-depth research efforts.

In conclusion, despite of many aspects that are to be improved, the modification of SiN cantilevers by MWCNTs coupled with the dynamic mode detection is an original concept that has never been raised previously. Furthermore, it should be noted that this technique could be further studied with bio-molecule markers for clinical diagnostics.
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