

Carbon nanotube-based Labels for Sensitive Nucleic Acids Detection

A.C.Lee*, J.S. Ye*, S.N. Tan**, D. Poenar***, F.S. Sheu*, C.K. Heng**** and T.M. Lim*

*Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543, dbsltm@nus.edu.sg, g0301209@nus.edu.sg

**Academic Group of Natural Sciences and Science Education, Nanyang Technological University, 1 Nanyang Walk, Singapore 637616

***School of Electrical and Electronic Engineering, Nanyang Technological University, Nanyang Avenue, Singapore 639798

****Department of Paediatrics, National University of Singapore, 5 Lower Kent Ridge Road, Singapore 119074

ABSTRACT

A novel multiple-labelled conjugate with carbon nanotubes (CNTs) to amplify hybridization signal was developed to improve the nucleic acids assay sensitivity. The carboxylated CNTs were functionalized by horseradish peroxidase (HRP) and single-stranded oligonucleotides via diimide-activated amidation. Oligonucleotides with specific sequence from SUP-B15 cell line that serves as molecular signatures of human acute lymphocytic leukemia (ALL) was synthesized and used as targets. Results show that both HRP and oligonucleotides were conjugated to the CNTs. The resulting conjugates can hybridize to its complementary targets and function as a label for nucleic acid quantification. These labels are also able to amplify the signals generated by the colorimetric method that significantly enhance the detection limit of the target by at least 1000 folds compared with conventional labels. These labels offer a unique advantage. Visual inspection of target detection was made possible due to the formation of visible aggregates.

Keywords: carbon nanotube, nucleic acid detection, multiple-label, colorimetric, disease diagnosis

1 INTRODUCTION

Nucleic acid detection has become increasingly important as we improve our understanding of the genetic basis of disease. The concentration of genetic targets is often low in the biological samples; therefore highly sensitive assays are essential for disease diagnosis.

Nucleic acid detection relies mainly on the specific hybridization of single-strand DNA to its complementary targets. This molecular recognition event triggers a usable signal, which can be an electrochemical, optical or mass readout depend on the method of signal transduction [1].

There are attempts to measure the hybridization signal without the presence of labels. However, these strategies generally achieve moderate sensitivity with the detection limit at the range of nM to sub-pM [1, 2]. Many types of nucleic acid assays developed thus require a secondary

detection technology, e.g., a label, which leads to hybridization signal amplification because a nucleic acid does not have strong intrinsic signal that enable ultrasensitive detection [3]. These label-based detection methods can easily achieve detection limit of pM to aM [4-8]. Therefore, signals generated by the labels are a key determinant of sensitivity for nucleic acid detection.

Several strategies have been developed for ultrasensitive assays, such as improved labels, multiple labeling and background noise reduction [3]. Multiple-label methods [9-11] show excellent sensitivity to detect as low as zeptomoles of target DNA [10]. However, these existing multiple-label methods have their drawbacks. These signal amplification methods especially those employing multi-layered DNA constructs and branched DNA generally involve series of steps to achieve the high degree of labeling, which may increase the complexity of assays. This drawback is mainly due to the lack of a material capable for carrying multiple labeling molecules.

Since its discovery, CNTs are of great interest for wide range of applications [12-18] owing to its unique properties. CNTs as sensing material/surface for nucleic acids detection has been widely studied [19-21]. However, its potentials as label in detection technology are yet to be explored.

Unique properties of CNTs such as high surface area, high aspect ratio and light weight are ideal as carrier for multiple labels. Combining these properties with the catalytic ability of HRP enzymes and the specificity of molecular-recognition features of nucleic acids, we therefore propose a novel CNT-based label for signal amplification of single binding event to improve the nucleic acids assay sensitivity.

2 EXPERIMENTAL

The carboxylated single-walled carbon nanotubes (SWNTs) were used for the label synthesis. The SWNTs are covalently functionalized with HRP enzymes via the single-stranded oligonucleotides (ssDNA), which serve as the detection probe for the hybridization assays. The functionalization is based on the diimide-activated amidation of SWNT-bound carboxylic acids groups with the amino-terminal of ssDNA in the presence of coupling reagent 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC).

The morphology of synthesized CNT-based labels was characterized using tapping-mode atomic force microscopy (AFM). The samples were adsorbed on mica substrate followed by rinsing with MilliQ water and dried with liquid nitrogen stream prior to measurement. Both height and amplitude images were recorded. Samples containing bare carboxylated SWNTs and freely suspended HRP were also analyzed.

The ability of synthesized CNT-based label functions as label for nucleic acid detection was verified by hybridization test. Sandwich DNA hybridization assay was performed on Dynal® streptavidin-coated magnetic bead platform. Oligonucleotides with specific sequence from SUP-B15 cell line that serves as molecular signatures of human acute lymphocytic leukemia was synthesized and used as targets. The sequences of the oligonucleotides used were:-

Capture probe: 5'-CCA TCG TTG GGC CAG ATC Biotin-3'

Detection probe: 5'-NH₂-TTC TGC GTC TCC ATG GAA-3'

Complementary target: 5'-GAT CTG GCC CAA CGA TGG CGA GGG CGC CTT CCA TGG AGA CGC AGA A-3'

Non-complementary target: 5'- AGT TCC ACC TCG TAT GTT ACT CCC AAG GAC TTT GAA AGC TTA AAG G-3'

The signal amplification of hybridization event by the CNT-based label is compare with the conventional HRP label. These conventional labels were made by conjugating HRP directly to the detection probe without SWNTs. HRP signal from hybridization complexes was measured by colorimetric method using 3,3',5,5'-tetramethylbenzidine (TMB) as substrate. The color product was quantified at wavelength 450nm.

3 RESULTS AND DISCUSSION

3.1 AFM Characterization

Figure 1b clearly shows the HRP-SWNT conjugate surrounded by free HRP molecules. The image suggests that the HRP molecules are immobilized as monolayer along the SWNT tube via the association with carboxylic acids at defect sites. No such layer of coating was observed in bare SWNTs sample (Fig. 1a). The single-stranded detection probes was not observed in these analysis probably due to its' small size scale of 18-mer which theoretically equivalent to about 6nm and sub-nm thinness based on Watson - Crick Model. The direct observation of HRP-SWNT conjugate using AFM again shows the evidence of the presence of multiple-HRP conjugated CNT-based label.

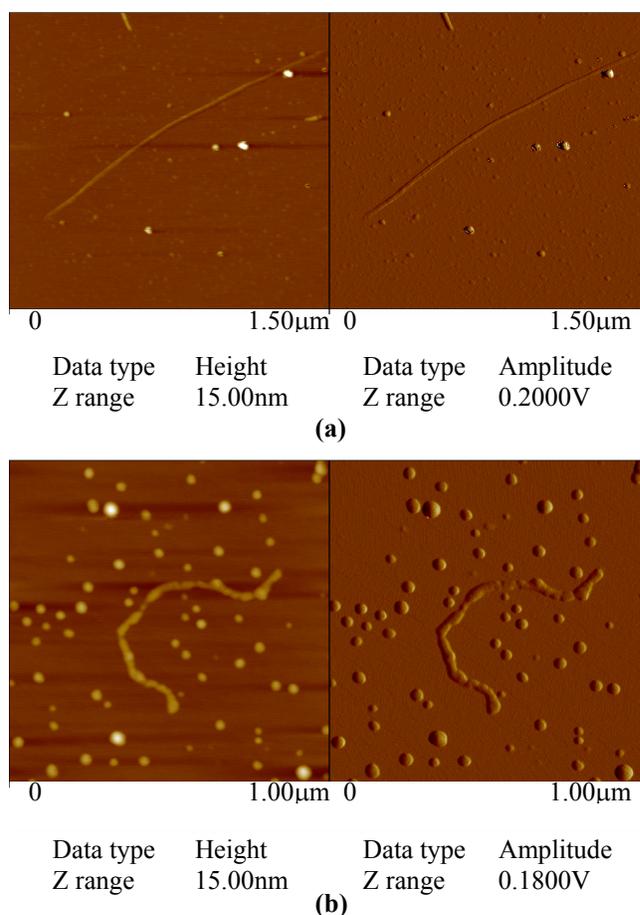


Figure 1: Height (left) and amplitude (right) images from the AFM analysis of (a). bare SWNT, (b). SWNT-HRP-detection probe conjugate sample on mica substrate.

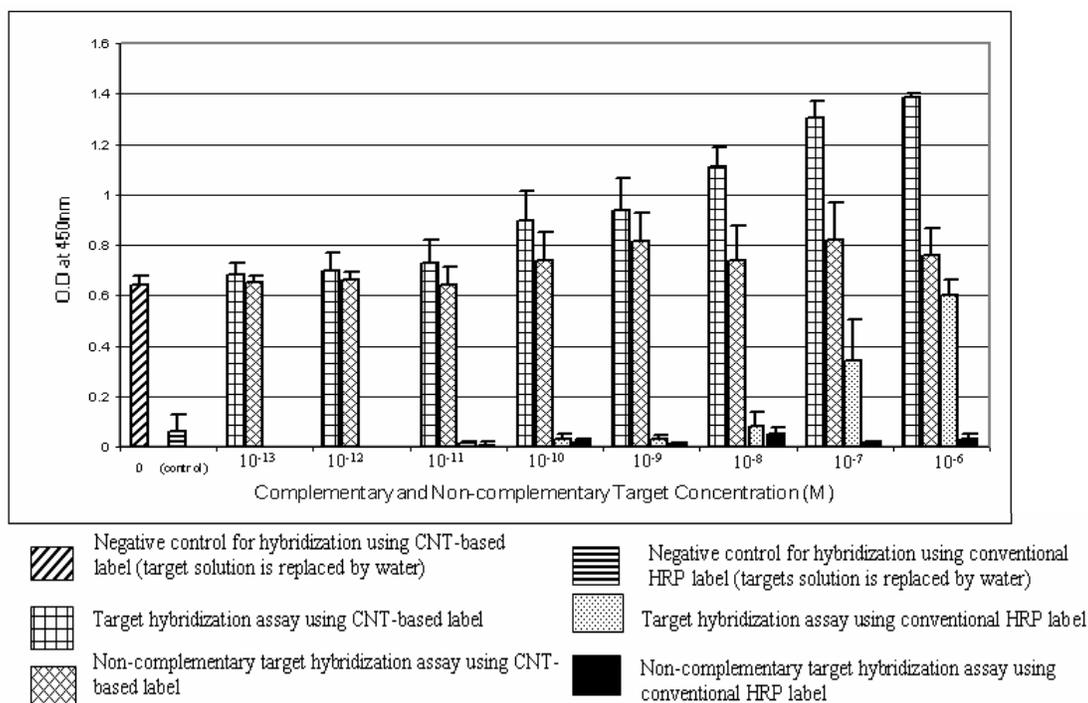


Figure 2: Nucleic acid hybridization detection using CNT-based label as compared with the conventional HRP label.

3.2 Nucleic Acid Hybridization and Detection

Figure 2 shows the results of nucleic acid hybridization detection using the CNT-based label as compare with the conventional HRP label. The target hybridization signal using CNT-based label is concentration dependent. The measured O.D. values increased with the increase of complementary target concentration. These hybridization signals were significantly stronger than that of conventional HRP label at every concentration being studied. Such responses suggest the presence of multiple-HRP conjugated CNT-based label and ssDNA detection probe complexes, which are able to hybridize with its complementary targets.

The signal amplification by the developed CNT-based label is clearly revealed even at low nucleic acid concentration ($<10^{-7}$ M) (Fig. 2). This newly developed CNT-based label showed strong intrinsic signal detected at the target concentration as low as 10^{-10} M whereas the conventional HRP label could only achieve 10^{-7} M. The detectable target hybridization signal at low target concentration (10^{-10} to 10^{-8} M) can only be the result of high degree labeling of a single hybridization event by the multiple-HRP conjugated CNT-based label; which had amplified the signal of a single hybridization event that was not achievable by conventional HRP label. The signal amplification by the novel label holds promise for detection limit to go below 10^{-10} M.

The beads appeared in darker color or as dark patches and dots (Fig. 3a to e) compare with their original color in light brown after hybridization of the CNT-based labels

with the targets. Such appearance is also concentration dependent, i.e. the darken appearance reduces with the decrease of target concentration. This observation is in good agreement with the quantitative results in Figure 2.

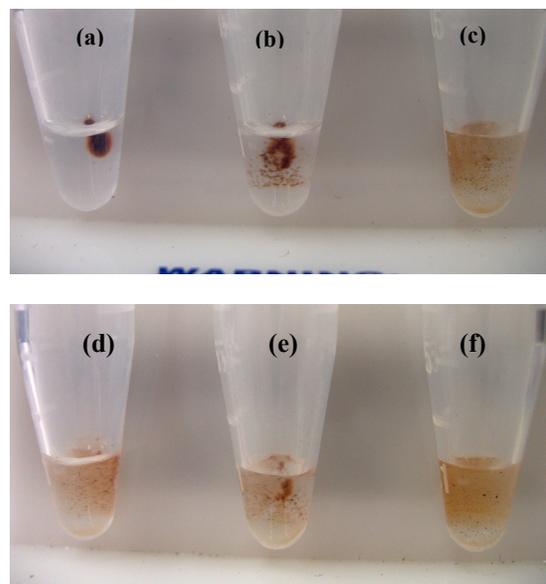


Figure 3: The appearance of the magnetic beads in wash buffer solution after hybridization assay using CNT-based label. Equal amount of beads and labeled detected probes was used in each tubes containing different concentration of complementary target DNA (a). 10^{-7} M; (b). 10^{-8} M; (c). 10^{-9} M; (d). 10^{-10} M; (e). 10^{-11} M and (f). negative control, i.e. targets are replaced by water.

The CNT-based labels are captured and accumulated on the capturing surfaces i.e. magnetic beads by means of the successful hybridization events. Accumulation of the CNTs comprising labels ultimately results in the formation of dark aggregates, which can be correlated with the amount or concentration of targets. The aggregates formed are visible in dark color as compare with the original color of magnetic beads. The aggregation phenomenon is still being studied.

Existing labels have not possessed such characteristic [21, 22] in which targets can be detected simply with visual inspection. No additional downstream signal development steps are required. This obviates the need for expensive and sophisticated detection systems.

4 CONCLUSIONS

In conclusion, the results prove that HRP and detection probes are successfully conjugated to SWNTs via covalent linkage. The resulting complex can hybridize to its complementary target and function as a label for nucleic acid detection. These labels are also able to amplify the signals generated by the colorimetric method that significantly enhance the detection limit of the target by at least 1000 folds compared with conventional labels. The label offers a unique advantage. Visual inspection of target detection was made possible due to the formation of visible aggregates. No additional downstream signal development steps are required. This obviates the need for expensive and sophisticated detection systems. Overall, the results reveal that CNTs are potentially an ideal solid phase platform for multiple labeling methods. The approach demonstrates great potential for ultrasensitive nucleic acid detection; as well as enables development of low cost visual detection based diagnostic device.

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