CARBON NANOTUBES-BASED LATERAL FLOW BIOSENSOR FOR SENSITIVE AND RAPID DETECTION OF DNA SEQUENCE

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MASTER OF SCIENCE

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ABSTRACT

Sensitive detection for DNA has drawn great attention in plenty of different areas such as genetics therapy and basic discovery research. Recently, nucleic acid lateral flow biosensor (NALFB) has gained considerable attention for DNA analysis. Compared with the traditional immunoassays, NALFB has these advantages: short assay time and a low cost. In this thesis, we report a carbon nanotubes (CNTs)-based NALFB for rapid and sensitive detection of DNA. Amine-modified DNA detection probe was covalently immobilized on the CNTs via diimide-activated amidation between the carboxyl groups on the CNTs surface and amine groups on the detection DNA probes. Sandwich-type DNA hybridization reactions were performed and the captured MWCNTs on test zone and control zone produced the characteristic black bands. Based on the catalytic property of CNTs to enhance the Chemiluminescence intensity of the reaction between hydrogen peroxide and Lumigen APS-5, a rapid detection of DNA sequence with high sensitivity is achieved.
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LIST OF ABBREVIATIONS

Ab.......................................................Antibody
APS-5.................................................Chemiluminescence substrate
BSA....................................................Bovine serum albumin
CL ......................................................Chemiluminescence
CNTs ..................................................Carbon nanotubes
DNA...................................................Deoxyribonucleic acid
EDC....................................................1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
ELISA ................................................Enzyme-linked immunosorbent assay
GNPs ..................................................Gold nanoparticles
LFA ....................................................Lateral flow assay
LFB ....................................................Lateral flow biosensor
LFSB..................................................Lateral flow strip biosensor
MES ...................................................2-(N-morpholino)ethanesulfonic acid
MWCNTs............................................Multi-walled carbon nanotubes
NALFB ..............................................Nucleic acid lateral flow biosensor
NHS-sulfo ..........................................N-Hydroxysuccinimide
ODN ...................................................Oligodeoxynucleotides
PBS ....................................................Phosphate buffer saline
PBST ..................................................PBS with 0.05% Tween 20
PCR ...................................................Polymerase chain reaction
POCT ................................................Point-of-care testing
Qdot....................................................Quantum dot
SiNRs ................................................. Silica nanorods
SSC .................................................... Sodium Saline Citrate
SWCNTs ............................................ Single-walled carbon nanotubes
Tris-HCl ............................................. Tris(hydroxymethyl)aminomethane hydrochloride
1. INTRODUCTION

Nowadays, nucleic acid detection has attracted more and more attention for the diagnosis and treatment of genetic diseases,\textsuperscript{1} infectious agents,\textsuperscript{2} bio-warfare agents,\textsuperscript{3} and drug discovery.\textsuperscript{4} The most famous method for DNA detection is called the polymerase chain reaction (PCR) due to its exponential amplification capability.\textsuperscript{5-6} Yet, even a small amount of the contamination can result in huge false positive. Moreover, the PCR-based detection strategy takes long period of time and requires a relatively clean environment, sophisticate and expensive instruments, which make it not suitable for rapid on-site test. Therefore, great efforts have been made to explore DNA biosensors over the past decade. The Point of care diagnostics which can provide near patient testing in a clinic, doctor’s office, or home can prove advantageous when rapid response is required or when suitable facilities are unavailable. Compared to equivalent methods used in laboratories, point of care testing is more affordable, as it eliminates the need for expensive instrumentation and skilled labor. One option involves the use of lateral flow assays. Pre-fabricated strips of dry reagents activated upon fluid application are already used in diagnostics, such as to ascertain pregnancy. Nucleic acid based detection assays on lateral flow offer several advantages over traditional microbiological detection.

1.1. Methods of DNA detection

There are numerous DNA detection methods besides the PCR. Another popular method is called: DNA Microarrays.\textsuperscript{7} The size of microarrays is similar to a microscope slide, or even smaller. Each spot on a microarray contains several identical DNA strands. The DNA sequence on each spot is unique. Each spot represents one gene. Thousands of spots are arrayed in orderly rows and columns on a solid surface (usually glass). The precise location and sequence of each spot is recorded in a computer database.
Colorimetric DNA detection method is one method which can provide visual detection. One literature reported a free, red-colored ssDNA-functionalized gold nanoparticles (GNPs) freely move across the semi-permeable cellulose acetate membrane. Another ssDNA-functionalized White-colored latex microparticles, on the other hand, are too large to pass through that barrier. Half of the ssDNA target is complementary to the ssDNA mobilized on GNPs and the rest ssDNA target sequence is complementary to the ssDNA bound to latex microparticles. In the presence of an ssDNA-target, target will hybridize simultaneously with two DNA on GNPs and latex microparticles therefore GNPs will bind latex particles, generating large-size, red-colored conjugates, which become retained by the membrane.

Molecular beacons can be used to detect DNA by fluorescence. MBs are single-stranded ODN probes that possess a stem-and-loop structure. The loop portion is complementary to the target. A fluorophore and a quencher are linked to the two ends of the stem. In its native state, the probe is a hairpin, and the two ends of the MB are in close proximity. Consequently, the fluorescence of the fluorophore is quenched by energy transfer. The hairpin stem is less stable than the binding between the loop and the target. In the presence of the target, the MB undergoes a conformational reorganization because the loop hybridizes with the target. The structure is opened, separating the fluorophore and the quencher. In this case, the MB emits an intensive fluorescent signal.

1.2. Nucleic acid lateral flow biosensor (NALFB)

Lateral flow biosensor (LFB) is a technique that is used to detect proteins, small molecules and viral antigens. This technique enables the rapid POC (point of care) diagnosis of infections and diseases like malaria, dengue fever and HIV. The lateral flow biosensor technique also detects cardiac markers, cancer etc. The lateral flow format uses a sandwich-like structure
such as that used in immunoassay: it has two antibodies that are bound to perform sandwich-style analysis. One antibody, mAb, is bound, initially non-covalently, in a horizontal stripe on a narrow strip of nitrocellulose. The nitrocellulose is void of protein to avoid vague observance of analyte and other proteins. The analyte and a second labeled antibody (classically, it is labeled with colloidal gold) are permitted to stream up nitrocellulose.

Carter and Cary (2007) have identified a method to detect DNA using LFAs. Lateral flow detection of RNA or DNA amplification reaction products offers a method of simplifying detection of nucleic acids. Dineva et al. (2005) has mentioned that lateral flow devices have been fabricated predominantly using more than one capture line, permitting more than one analyte. As a step toward improving information detection of lateral flow nucleic acids, the researchers have developed nitrocellulose patterning methods that allow microarray characteristic densities to be met on compatible substrates of lateral flow. Thus, the lateral flow microarray method develops sequence-specific detection, opening the door to increasingly multiplexed implementations for a vast number of assays, well-suited for point of care and other field applications.

1.3. Principle of NALFB

NALFB is processed on a lateral flow strip biosensor (LFSB), which is composed of porous membranes, recognition elements, and a signal-generating system (commonly colored particles as the labels). The LFSB is composed of four components: sample pad, conjugate pad, nitrocellulose membrane and absorption pad. The movement of liquid along the strip is essential in the LFSB, thus each part overlaps onto one another to ensure the migration. One end of the strip is located a sample pad which is usually made of cellulose for loading sample solution (e.g. target DNA). The conjugate pad, made from glass fiber, is attached with the sample pad; colored
particles labeled with recognition elements are dispensed/pipetted (e.g. CNTs-DNA conjugate), and dried on the conjugate pad. The nitrocellulose membrane acts as detection zone with sprayed at least two lines: a test line and a control line; the test line is used to recognize the sample analyte and capture the colored particles to generate the detectable signal, while the control line is used to validate the proper performance of the strip. The attached absorption pad at the other end of the strip is to maintain the flow of the liquid since the capillary force of the strip material is the driving force for the movement of the liquid.

1.4. Labels used in lateral flow assay

Labels used in LFA are essential in the development of LFA which can improve its sensitivities. The intensity of test line depends on the amount of captured labels in the test zone or its catalytic effects. The ideal label for LFA would obtain several advantages such as good stability, wide detection dynamic range and high sensitivity, easy conjugation and low cost. Various labels including nanotubes (Carbon nanotubes), nanoparticles, nanowires, dye-doped microbeads and hybrid nanocomposites have been utilized in LFA.

1.4.1. Carbon nanotubes (CNTs)

Carbon nanotubes (CNTs), as a typical one-dimension nanomaterial, have been successfully demonstrated as sensitive electrochemical biosensors as a result of their unique physical, chemical, and electrical properties.\textsuperscript{13-17} Aptamer-modified CNTs field-effect transistors were fabricated for the detection of thrombin and immunoglobulin E.\textsuperscript{18} Regarding electrochemical aptasensors, amperometric aptasensors based on CNT-based nanocomposites have received the most attention. A simple and efficient post labeling strategy, based on dye-induced peeling of the aptamer molecules from single-walled CNTs (SWCNTs), was used to develop an aptasensor for thrombin detection.\textsuperscript{19} CNTs or their composites show the capacity to
function as a signal amplifier by facilitating aptamer probe immobilization and improving the electrochemical properties of the transducer as a result of their tremendous surface-to-bulk ratios and excellent electrocatalytic performance. The mainly difference between MWCNTs and SWCNTs is that MWCNTs contains larger surface area that would potentially immobilize more DNA probe or protein therefore offer higher DNA probe density on the CNTs surface and higher hybridization efficiency during the test. Thus MWCNTs is chosen as the DNA carrier rather than SWCNTs.

1.4.2. Gold nanoparticles (GNPs)

As one of the most popular label in LFA, gold nanoparticles (GNPs) had several advantages such as its easy preparation and conjugation, bright color for visualization, good stability and cheaper cost. Large number of tests have been done based on GNPs labels for detecting infectious agents, metabolic disorders, toxic compounds. The qualitative or semiquantitative detection of analytes would be realized by the visualization with the analysis of color intensity. However, the detection limit and sensitivity of GNPs-based LFA remains to be improved, particularly for the detection of trace amount of analytes. Another issue from the GNPs-based LFB is the aggregation of GNPs–DNA conjugates during the preparation and assay test.

1.5. Colorimetric analysis

Colorimetric detection is an assay developed for the detection of a target molecule, indicating the presence of the target with a color change phenomenon. The target molecule can be anything ranging from a protein, DNA, cell to even metal ions. For quantitative measurements, the optical intensities of both test and control lines on the biosensor were recorded simultaneously by using a strip reader combined with the ‘GoldBio strip reader’
software. The numeric values of recorded data were obtained from pixel images of test and control lines. The principle of colorimetric analysis here is trying to use Analyzer DT 1030 to read color intensity of the black band of CNTs on the Lateral Flow Biosensor.

![Analyzer DT 1030](image)

**Fig. 1.1.** Analyzer DT 1030 is used to read color intensity of the black band on the Lateral Flow Strip Biosensor

### 1.6. Chemiluminescence analysis

Chemiluminescence is the generation of electromagnetic radiation as light by the release of energy from a chemical reaction. Chemical reactions using synthetic compounds and usually involving a highly oxidized species such as peroxide are commonly termed Chemiluminescence reactions. The Chemiluminescence used in this study is a reaction between a Chemiluminescence
substrate called APS-5 and hydrogen peroxide, without a strong oxidant Chemiluminescence intensity of APS-5 is very low, Chemiluminescence intensity will dramatically enhance if an oxidant such as H₂O₂ presents. Figure. 1.2. shows the reaction between APS-5 and H₂O₂, first of all the H₂O₂ will oxidized the double bond of APS-5 to form an intermediate which is under excited status and not stable, it will release energy in terms of light and form a ketone structure finally that is stable.

Fig. 1.2. Chemiluminescence reaction between Lumigen APS-5 and H₂O₂

1.7. Objective

My research project tries to use MWCNTs as DNA carrier as well as its visual detection to show the black band on later flow biosensor to check if there’s target DNA presents. The catalytic property from MWCNTs is used to significantly enhance the Chemiluminescence reaction between APS-5 and H₂O₂. Based on this principle, with more target DNA presents, more MWCNTs will be successfully immobilized onto the test line, a higher Chemiluminescence intensity could be obtained.
2. CARBON NANOTUBES-BASED LATERAL FLOW BIOSENSOR FOR SENSITIVE AND RAPID DETECTION OF DNA SEQUENCE

2.1. Introduction

Different transducers have been applied in connection with DNA biosensors (electrochemical, optical, acoustic, piezoelectric, etc.,) have been reported in the literatures.\(^{27-29}\) Recently the application of nanomaterials (nanoparticles, nanowire and nanotubes) for sensitive detection of DNA becomes more and more popular and draws more and more attention.\(^{30-33}\) Even copies of DNA or RNA can be detected without PCR amplification.\(^{34-38}\) Yet, the applications of DNA biosensors have been limited because of expensive cost, complex operations and requirement of trained personnel.\(^{39}\) Recently, nucleic acid lateral flow biosensor (NALFB) has attracted considerable attention due to its rapid, portable and low-cost characteristics.\(^{40-41}\) NALFB have gained an increasing attention for performing fast and low-cost analysis of samples at the place where the samples are collected. Compared to traditional laboratory analysis platforms, such a lateral flow assay or biosensor has some advantages for point-of-care or in-field testing: short assay time, small volume of sample, user-friendly and low cost. The NALFB have been used for clinical diagnosis or screening of diseases, testing drugs of abuse, monitoring the safety of water and food. While conventional NALFB is based on colloidal gold and latex or polystyrene beads for visual detection through the color formation, thus this approach only allows qualitative or semi-quantitative analysis. The application of NALFB is challenged for detection of trace amount analytes. Therefore, it is highly desirable to develop an ultrasensitive NALFB for detection of DNA. Chemiluminescence is a sensitive measurement tool and has been applied in life sciences, clinical diagnosis, environmental and food analysis. Such high sensitivity is due to the signal amplification from catalytic reaction in the presence of
carbon nanotubes to enhance the Chemiluminescence reaction between APS-5 and hydrogen peroxide. In addition to the wide dynamic range of the Chemiluminescence detection ensures the analysis of samples with a broad range of concentrations. In addition, Chemiluminescence detection due to the absence of an excitation source enhances the detectability compared to fluorescence measurement. Recently Chemiluminescence detection has been used for LFB with improved detection sensitivity with respect to colloid gold or latex beads based LFB.

2.2. Experimental section

2.2.1. Apparatus

Airjet AJQ 3000 dispenser, Biojet BJQ 3000 dispenser, Clamshell Laminator and the Guillotine cutting module CM 4000 were from Biodot LTD (Irvine, CA). A portable strip reader DT1030 was purchased from Shanghai Goldbio Tech. Co., LTD (Shanghai, China). QuantiReader™ Benchtop Luminometer was bought from DiaCarta (Hayward, CA) for Chemiluminescence detection.

2.2.2. Reagent and materials

Carboxylated multi-walled carbon nanotubes (MWCNTs,purity > 95%), streptavidin, bovine serum albumin (BSA), N-(3- Dimethyl amino propyl)-N’ ethyl carbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimide (sulfo-NHS), 2-(4-Morpholino)ethanesulfonic acid (MES), sucrose, Tween20, phosphate-buffered saline (0.01M PBS, pH7.4), hydrogen peroxide (H₂O₂) and sodium chloride-sodium citrate(SSC) buffer 20 concentrate (pH 7.0) were purchased from Sigma-Aldrich (St.Louis, MO). Lumigen APS-5 was purchase from Lumigen (Southfield, Michigan). Glass fibers (GFCP000800), cellulose fiber sample pads (CFSP001700), laminated cards (HF000MC100) and nitrocellulose membranes (HFB24004) were provided from Millipore (Bedford, MA). All other chemicals were of analytical reagent grade. All buffer solutions were prepared using ultrapure (>18 MΩ cm) water from a Millipore Milli-Q water
purification system (Billerica, MA). All the DNA oligonucleotides used in this study were purchased from Integrated DNA Technologies, Inc. (Coralville, IA) and had the following sequence:

Target DNA: 5′-ATGACCTATGAATTGACAGAC-3′.
Amine-modified detection DNA probe: 5′-Amino-GTCTGTCACA-3′.
Biotinylated capture DNA probe: 5′-ATAGGTCAT/Biotin-3′.
Biotinylated control DNA probe: 5′-Biotin/MC6-D/TTGACAGAC-3′.

2.2.3. Preparation of streptavidin-biotinylated DNA conjugates

Two hundred microliter of 2.5 mg/mL of streptavidin was mixed with 50 nmol biotinylated capture or control DNA probes. Then this mixture was incubated on shaker for 1h. After that adding 500 μL PBS into the mixture, the solution was centrifuged with centrifugal filter for 20 min at 6000 rpm at 4 °C. Repeat this step three times. The remaining solution in filter was diluted to 600 μL by using PBS.

2.2.4. Preparation of shortened multi-walled carbon nanotubes

The carboxylated MWCNTs were firstly treated with mixed concentrated acids (H₂SO₄:HNO₃, 1:3) under ultrasonication for 3 hours, and washed with water for three times.

2.2.5. Preparation of carbon nanotubes-DNA conjugates

Conjugates are prepared based on the following procedures. The MWCNTs (0.5mg) was treated with 9.6mg-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and 5.43mg N-Hydroxysuccinimide (sulfo-NHS) in 1.0 mL MES buffer (0.1M, pH = 4.7). After shaking at room temperature for 15min, the mixture was washed by centrifuging at 12,000 rpm for 5min. Discard the supernatant and resuspend the pellet in PBS buffer. Repeat the above operations/treatments three times to remove the excess reagents. The amine-modified DNA
detection probe was then added to the activated MWCNTs solution with a final concentration at 0.3OD mL$^{-1}$ and the solution was incubated over night at room temperature. After overnight incubation, this mixture was centrifuged at 10000 rpm for 20min. The supernatant was discarded and the pellet was resuspended in PBST. After repeating the above step 3 times, the pellet was resuspended in 1 mL eluent buffer containing 20mM Na$_3$PO$_4$·12H$_2$O, 5% BSA, 10% sucrose, and 0.25% Tween-20. The CNTs–DNA conjugate solution was stored at 4°C before further use.

2.2.6. Sample assay procedure

One hundred microliter of sample solution containing different concentration of target DNA in running buffer (1/4 SSC, 2% BSA) was added onto the sample pad, then the solution migrated toward absorption pad. The test and control zones could be evaluated visually within 30min. For quantitative measurements, the Chemiluminescence intensities between and APS-5 and H$_2$O$_2$ is evaluated by DiaCarta (Hayward, CA) Chemiluminescence reader, as a result of the presence of MWCNTs, the reactions between APS-5 and H$_2$O$_2$ could be significantly catalyzed, based on this principal, with more target DNA presents, more CNTs will be immobilized onto the test line, therefore the catalytical effect of CNTs to enhance the Chemiluminescence intensities will be much larger. The digital images of the NALFB were obtained with Nikon COOLPIX 4200 camera (Nikon, Japan) and then transferred to a computer.

2.2.7. Colorimetric detection method

The principal of colorimetric detection method is to measure the black color intensity on the test line, with more target DNA presents, more CNTs could be accumulated onto the test line, variation amount of target DNA will immobilize different amount of CNTs to the test line therefore the colorimetric intensity will be different. Figure 2.1. shows the cuvette which contains lateral flow strips and it can make both test line and control line visible, after strips
settle into cuvette, this cuvette will be place into Analyzer DT 1030 to read those color intensities.

**Figure 2.1.** Lateral flow strips placed into cuvettes and the color intensity reader: Analyzer DT 1030.

### 2.2.8. Chemiluminescence detection method

The principal to use Chemiluminescence method to detect the concentration of DNA is based on the catalytic property of CNTs to significantly enhance the Chemiluminescence intensity of a reaction between APS-5 and H$_2$O$_2$, if more target DNA presents more CNTs will be immobilized onto the test line, therefore the catalytic effects from CNTs will become larger. Based on this principal, after the sample assay procedures, the test lines are obtains. Figure 2.2. shows the procedures to obtain the Chemiluminescence intensity. Scissors is used to cut the test line part of the lateral flow strip hence the CNTs is obtained. Then place this test line into one clean cell of 64 well plate, add the mixture of H$_2$O$_2$ and Lumigen APS-5 then place this 64 well plate into DiaCarta (Hayward, CA) Chemiluminescence instrumentation, finally use the software on the computer to read corresponding cell’s Chemiluminescence intensity.
2.3. Results and discussions

2.3.1. Principle of Chemiluminescence lateral flow strip biosensor

Fig. 2.1. illustrates the principle of DNA measurement on the MWCNTs based NALFB. Conjugate DNA probe is complementary with the control line DNA probe. Half of the target DNA is complementary with the conjugate DNA probe and the rest half of the target DNA is complementary with the test line DNA probe. Typically, the sample solution containing target DNA was applied to the sample pad. Subsequently, the solution migrated by capillary action, then the hybridization reactions between target DNA and the detection DNA probe (conjugate DNA probe) of MWCNTs–DNA conjugates occurred, and the formed complexes (MWCNTs–DNA–target DNA) continued to migrate along the strip. After reaching the test zone, the complexes were captured by the biotinylated capture DNA probe immobilized on the test zone via the second hybridization reaction. A characteristic black band could be observed because of the accumulation of MWCNTs on the test zone. Once the solution passed through the control zone, the excess MWCNTs-DNA conjugates which didn’t successfully bind to the target DNA probe were captured by the biotinylated control line DNA probe, thus a second black band...
appeared. In the absence of target DNA, only the black band in the control zone will appear.

Based on the characteristic property of CNTs which can significantly catalyze Chemiluminescence reaction between Lumigen APS-5 and \( \text{H}_2\text{O}_2 \), quantitative analysis is realized by analysis the intensities of Chemiluminescence which applied only the test line part of the strip to enhance the intensity of the reaction between Lumigen APS-5 and \( \text{H}_2\text{O}_2 \), with more target DNA presents, more MWCNTs–DNA conjugates can be immobilized onto the test zone and more carbon nanotubes will present, therefore the Chemiluminescence can be significantly enhanced based on the principle of CNTs can catalyze the Chemiluminescence of the mixture of Lumigen APS-5 and \( \text{H}_2\text{O}_2 \).
Fig. 2.3. Schematic illustration of the principle of DNA measurement on MWCNTs-DNA conjugate based lateral flow biosensor.

2.3.2. Optimization of experimental parameters

To obtain the best performance of MWCNTs-based NALFB, analytical parameters, including running buffers, the percentage of SSC, the concentration of the amine-modified detection DNA probe for preparing MWCNTs–DNA conjugates, the number of capture DNA probes on the test zone, and the volume of the MWCNTs–DNA on the conjugate pad, dilution percentage of $\text{H}_2\text{O}_2$, dilution times of the mixture of Lumigen APS-5 and hydrogen peroxide, loading amount of Lumigen APS-5 and $\text{H}_2\text{O}_2$ mixture were optimized. First, we studied the
effect of running buffers on the S/N ratio of NALFB. Sample solutions were prepared by diluting the target DNA stocking solution with different buffers. Fig. 2.4. shows the histogram for the S/N ratios of NALFB via the buffers. It can be seen the highest S/N ratio was obtained with 1/4 SSC + 2% BSA, which was then used as running buffer in the following NALFB.

![Bar graph showing S/N ratios of different buffers.]

**Fig. 2.4. S/N of different buffers**

Secondly, the percentage of SSC inside the SSC + BSA buffer is optimized as well because different amounts of salt may affect the binding efficiency of DNA strands. In order to check the DNA hybridizing efficiency, several different portions of SSC were chosen to test. Fig. 2.5. presents the histogram for the S/N ratios of NALFB via the different percentage SSC buffers. From the figure it can be observed the highest S/N ratio was obtained with 12.5% SSC, which was then used as SSC% in the future detection. This percentage of SSC will give the best hybridizing efficiency of DNA strands.
The amount of detection DNA probe bond to the MWCNTs can affect the hybridization efficiency between the target DNA and detection DNA probe on MWCNTs, and thus affect the response of NALFB. We optimized the concentration of the amine-modified detection DNA probe when preparing MWCNTs–DNA conjugates. As seen from Fig. 2.6. the S/N ratio first increased until 0.3 ODmL⁻¹; a further increase in DNA’s concentration caused a decrease in the S/N ratio. The S/N ratio loss at a high concentration may be caused by stereo hindrance from the high density of detection DNA probe on the CNTs surface.
The amounts of captured DNA probes immobilized at the area of test line will affect the NALFB performance as well. The amounts of captured DNA was optimized by dispensing different amounts of streptavidin-biotinylated DNA probes, which was achieved by changing the dispensing cycles on the test zone. Fig. 2.7. presents the effect of the captured DNA amount on the NALFB’s S/N ratio. The highest S/N ratio was obtained with 3 dispensing cycles, which were then used in the linear curve detection NALFB. The decreased S/N ratio with more dispensing cycles resulted from the increased background signal.

**Fig. 2.6.** S/N of different concentration of detection DNA probe
Fig. 2.7. S/N of different dispensing times of test line

The intensity of test and control lines were greatly influenced by the amount of MWCNTs–DNA conjugates dispensed on the conjugate pad. To obtain the best S/N ratio, the MWCNTs-DNA on the conjugate pad was optimized by increasing the volume of the MWCNTs-DNA conjugates loaded on the conjugate pad. Fig. 2.8. presents the histogram for the S/N ratio of NALFB via the conjugate volume. The S/N ratio increased up to 6.0 micro liter; a further increase in volume gave a decrease S/N ratio. The S/N ratio loss at a larger volume may be attributed to a saturation of signal intensity and nonspecific adsorption increased. Therefore, 6.0μL of MWCNTs–DNA conjugate was used as the optimal volume throughout the entire research.
Fig. 2.8. S/N of different loading amount of MWCNTs-DNA conjugate

The volume ratio of the mixture of APS-5 and H₂O₂ will also significantly affect the results of Chemiluminescence detection. The best composition of the mixture can reduce the intensity of the background and enhance intensities when target DNA presents. As shown in Fig 2.9., when the volume ratio between APS-5 and H₂O₂ is 1:2, the best S/N could be achieved, other composition will either reduce the intensities when target DNA presents or caused higher background.
Fig. 2.9. S/N of volume ratio between Lumigen APS-5 and H$_2$O$_2$

The dilution percentage H$_2$O$_2$ is another essential factor of the Chemiluminescence detection. A good dilution percentage of hydrogen peroxide can reduce the intensity of the background and enhance intensities when target DNA presents. As shown in Fig 2.10., when the initial H$_2$O$_2$ (30% H$_2$O$_2$) is diluted 100 times, the best S/N could be reached, other composition will either caused higher background or reduce the intensities when target DNA presents.

Fig. 2.10. S/N of dilution percentage of H$_2$O$_2$
The loading amount of the mixture of $\text{H}_2\text{O}_2$ and APS-5 is another important factor of the Chemiluminescence detection. To get the best volume of the mixture which can reduce the intensity of the background and enhance intensities when target DNA presents, several different amounts are tested. As shown in Fig 2.11., when the volume of the mixture is 50 micro liter the best S/N could be obtained, other composition will either caused higher background or reduce the intensities when target DNA presents.

![Loading amount of Lumigen APS-5 and $\text{H}_2\text{O}_2$ mixture/μL](image)

**Fig. 2.11.** S/N of loading amount of Lumigen APS-5 and $\text{H}_2\text{O}_2$ mixture

Because some buffers may have negative effects to the Chemiluminescence reaction between APS-5 and hydrogen peroxide. Therefore, several different kinds of buffers are tested again through the Chemiluminescence reaction. As shown in Fig 2.12., SSC + 2% BSA is still the best buffer which can give the highest S/N, there for 1/8 SSC + 2% BSA will continue be used as the running buffer of this project.
Different buffers in Chemiluminescence detection

<table>
<thead>
<tr>
<th>Buffer</th>
<th>S/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSC + 2%BSA</td>
<td>8.99</td>
</tr>
<tr>
<td>Tris-HCl + 2%BSA</td>
<td>4.47</td>
</tr>
<tr>
<td>PBS</td>
<td>4.85</td>
</tr>
<tr>
<td>PBST</td>
<td>3.76</td>
</tr>
</tbody>
</table>

**Fig. 2.12.** S/N of different buffers in Chemiluminescence detection

### 2.3.3. Analytical performance of colorimetric detection method of lateral flow strip biosensor

After these parameters were optimized, we tested the performance of the MWCNTs-based NALFB in the presence of different concentrations of target DNA. Each sample was detected 3 times and the average value of three measurements was used to plot the calibration curve. **Fig. 2.13.** presents the typical photo images of MWCNTs-based NALFB after testing the different concentration of target DNA. No black band was observed in the blank test, indicating negligible non-specific adsorption under the optimized experimental conditions. The test line was quite visible when the target DNA concentration is 0.5nM, which can be used as a threshold for the visual detection of DNA. The resulting calibration curve shows that the colorimetric intensities versus the concentration of target DNA over the 0.1–20nM range, a good linear relationship with a detection limit of 1.04nM.
Fig. 2.13. Typical photo images (up) of the NALFB in the presence of different DNA concentrations and the resulting colorimetric calibration curve (middle: from left to right: Blank, 0.1nM, 0.2nM, 0.5nM, 1nM, 2nM, 5nM, 10nM, 20nM) and the resulting calibration curve (down).
2.3.4. Analytical performance of Chemiluminescence detection method of lateral flow strip biosensor

Under optimal experimental conditions, we examined the performance of the MWCNTs-based NALFB in the presence of different concentrations of target DNA. Each sample was also detected 3 times and the average value of three measurements was used to draw the calibration curve. Fig. 2.14. presents the typical photo images of MWCNTs-based LFB after testing the different concentration of target DNA. There was no band observed in the control test, indicating that we can ignore the non-specific adsorption under the optimized experimental conditions. The intensity of Chemiluminescence of the test line increased with the increase of target DNA concentration up to 20nM. The black band was quite visible, even in the presence of 0.1nM target DNA, which can be used as a threshold for the visual detection of DNA. The resulting calibration curve shows that a good linear relationship between the target DNA concentration and Chemiluminescence intensities within the range between 0.1 to 20nM, with a detection limit of 0.095nM.
Fig. 2.14. Typical photo images (up) of the NALFB in the presence of different DNA concentrations and the resulting Chemiluminescence calibration curve (middle: from left to right: Blank, 0.1nM, 0.2nM, 0.5nM, 1nM, 2nM, 5nM, 10nM, 20nM) and the resulting calibration curve (down).
2.3.5. Reproducibility

Except its high sensitivity, the MWCNTs-based NALFB also gave high reproducibility. The reproducibility of the NALFB was assessed by testing the NALFBs in the absence and presence of 5.0nM and 50nM target DNA. Samples at the same concentration levels were tested 6 times, the similar responses were obtained at the same concentration levels. The relative standard deviations (RSD) of the signal for control, 5nM and 50nM were 5.3%, 4.4%, and 9.6%, which indicates a good reproducibility of the measurements. The stability of the MWCNTs-based NALFB was also checked by storing the tested NALFB strips at room temperature for several months. It was found that their responses did not change dramatically. The RSD of the NALFB for 5nM of target DNA was less than 5% compared with that obtained with the newly prepared NALFB, indicating the MWCNTs-based NALFB has a good stability.

2.4. Conclusion

I have successfully developed MWCNTs-based NALFB for sensitive and rapid detection of DNA sequence. The sensitivity of the MWCNTs-based NALFB using Chemiluminescence detection method was enhanced 10 times compared to the colorimetric detection method. After systematic optimization, the MWCNTs-based NALFB was capable of detecting 0.095nM DNA. Moreover, the use of MWCNTs labels avoided the aggregation of conjugates, which was often met in the traditional gold nanoparticle-based NALFB. The MWCNTs-based NALFB thus open a new door to prepare a new generation of NALFB, and shows great promise for in-field and point-of-care diagnosis of genetic diseases and for the detection of infectious agents. The concept should be extended to visually detect protein biomarkers using the MWCNTs-based NALFB. Further work will aim to amplify the signal using enzyme-loaded MWCNTs and detect miRNA in cell-lysate and biological fluids.
3. SUMMARY

The research work described in this thesis demonstrated the improved sensitivity of NALFB based on using MWCNTs as the label and two different measurement strategies. The amplified response signals were obtained from NALFB combing with MWCNTs, Chemiluminescence detection methods. Those ultrasensitive biosensors broadened the application field of NALFB for detection of DNA at trace level which could not be analyzed using conventional LFSB method. The improved NALFB showed promising applications in early diagnosis of DNA-related cancer and detection of infectious disease.

The signal amplifications were investigated by these two strategies:

1. A colorimetric detection method using NALFB based on the colorimetric intensity of black bands of CNTs on the test line.

2. A Chemiluminescence detection method using NALFB based on the catalytic property of CNTs to enhance the intensity of the Chemiluminescence reaction between APS-5 and H$_2$O$_2$.

Even with highly enhanced sensitivity, the NALFB are still faced with numbers of challenges for point-of-care diagnosis or on-field application. The analytes tested within complex biological samples, such as human blood or saliva, is a greatly important future application. Moreover, to achieve an excellent specificity in validation of DNA for cancer development and progression, simultaneous analysis of multiplexed DNA strands with biosensor becomes particularly important in laboratory research and clinical diagnosis. Extensive experiments will be necessary to be carried out with analysis of patient samples for configuration of reliable detection platform. More works will also be focused on the multiplex detection on a single LFSB for diagnostics of the specific cancer-related disease.
4. REFERENCES


21. Zhang, G. P.; Guo, J. Q.; Wang, X. N.; Yang, J. X.; Yang, Y. Y.; Li, Q. M.; Li, X. W.; Deng,


