Colorimetric Detection of Polynucleotides on Polypropylene Slices

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The gold-label-silver-stain method (GLSS) for DNA hybridization detection has been receiving increased interest as a colorimetric detective method, demonstrating the advantages of non-radioactivity, non-quenching effect of fluorescence and simplicity for analytical equipment. A colorimetric detection based on the GLSS method was applied to DNA arrays *in situ* synthesized on polypropylene (PP) slices. In this paper a simple plasma treatment was employed to graft amino ($-NH_2$) on the polypropylene slice surfaces, where DNA probes were immobilized *via in situ* synthesis. Hybridization was accomplished by a sandwich hybridization format. With the amplification of Silver Enhancer Solution, the hybridization signals were recorded with a scanner. A target DNA concentration as low as 100 fM was detected. Complementary and mismatched sequences were clearly distinguished, and the ratio of the background-subtracted gray scale values for a perfect match, single-base mismatch, 2-base mismatch and 3-base mismatch is 22:16:9:4. The sensitivity of the *in situ* synthesis system was 3 orders of magnitude higher than that of the spotting system, and the signals of the former were about 2-times stronger than that of the latter under the same target DNA concentration.

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Introduction

Gene-chip technology based on various matrixes and multiplicate detective methods has received much attention in recent years because of its application in the diagnosis of pathogenic and genetic disease.¹ The gold label silver stain (GLSS) method developed three decades ago was first used in immune detection. In recent years it was applied to sequence-specific DNA detection, which has many desirable features, such as higher sensitivity and specificity compared with the fluorophore labeled method, as well as no radioactivity, no fluorescence quenching, and little or no required instrumentation.²⁻⁴ Mirkin's group developed GLSS on a slide to detect spotted DNA arrays, and exploited it for electrical detection, as well as assembled gold nanoparticles connected with DNA into network materials.³⁻⁶

Because of its low cost, chemical and mechanical properties, ready availability in a number of different forms, and the great potential of the grafting technique to create reactive centers, the polymer is an attractive matrix for DNA immobilization. Many papers have been published on the immobilization of protein and DNA using nylon as a support substrate.⁷⁻¹¹ To graft amino functions onto the surface of nylon, the reported chemical grafting methods are generally complicated. Here, polypropylene slices can be grafted by an amino ($-NH_2$) by a plasma treatment that is used as a DNA chip matrix.

The present work aims to develop colorimetric detection of polynucleotides on grafted polypropylene slices. The oligonucleotide-functionalized gold nanoparticles (gold-labeled probes) hybridize to target DNA that have been hybridized to

DNA probes *in situ* synthesized on the surface of PP slices, which was the so-called sandwich assay format.⁴ With the amplification of Silver Enhancer Solution, the hybridization signals were recorded with a scanner. Such a GLSS colorimetric detection method demonstrated a promising viability to DNA array-based profiling.

Experimental

Reagents

$H AuCl_4 \cdot 3H_2O$ was purchased from Shanghai Reagent Factory. MicroHyb hybridization buffer was purchased from Research Genetics. Alkanethiol-modified oligonucleotide, target oligonucleotide were purchased from Shanghai Shenyou Biotechnology Company. The sequences were as follows, respectively:

P1: 3'-TTTTCAAACCTCTCGA-SH-5'

T0: 5'-ACATCGATGCACTGAGTGAATGGAGGGCCGCA
AGAAAAAGTTTGAGAGCT-3'

T1: 5'-ACATCGAAGCACTGAGTGAATGGAGGGCCGCA
AGAAAAAGTTTGAGAGCT-3'

T2: 5'-ACATCGTAGCACTGAGTGAATGGAGGGCCGCA
AGAAAAAGTTTGAGAGCT-3'

T3: 5'-ACATCGTACCACTGAGTGAATGGAGGGCCGCA
AGAAAAAGTTTGAGAGCT-3'

The deoxynucleoside phosphoramidites used in *in situ* synthesis were purchased from Transgenomic. All other reagents were all of analytical grade.

Instrumentation

A transmission electron microscopy (TEM) image was recorded with a JEM-200CX TEM. A Beckman J-30I centrifuge was used for the centrifugation of an Au nanoparticle

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solution. The superpure water used was purified with a Milli-Q water purification system. DNA probes were *in situ* synthesized with an Expedite 8909 DNA synthesizer. Silver stained images were recorded with a scanner.

Preparation of Au nanoparticles

The 13 nm diameter Au particles were prepared according to Frens¹ method. All glassware was cleaned in aqua regia ($\text{HCl}:\text{HNO}_3 = 3:1$), rinsed with super-pure water, and dried. The 1% (w/w) $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ aqueous solution was prepared prior to use. Then 20.6 ml of the 1% HAuCl_4 solution and 480 ml of superpure water were brought to a flask equipped with a condenser and a heater. The solution was heated to reflux with vigorous stirring. Then, 50 ml of a 38.8 mM trisodium citrate solution was quickly added. The color of the solution changed to deep red in a few seconds. After the color change, the solution was brought to reflux for 15 min continuously, and allowed to cool naturally to room temperature after moving away from the heater. Subsequently, the solution was filtered through a $0.45 \mu\text{m}$ nylon membrane, then stored at 4°C in dark bottles. TEM determined the size of the nanoparticles (Fig. 1). The TEM indicated that the sizes of the nanoparticles were homogeneous, with a small deviation ($\sim 13 \pm 1 \text{ nm}$).

Preparation of 5'-alkanethiol-capped oligonucleotide-modified Au nanoparticles

The gold nanoparticle-labeled probes were derived from mixing 2 ml of the above-mentioned 13 nm diameter Au nanoparticle solution with 1 OD of (alkanethiol)oligonucleotide P1 (final oligonucleotide concentration is $3.51 \mu\text{M}$). After being incubated for 16 h at room temperature, the solution was brought to 3 ml of 0.1 M NaCl, 10 mM phosphate buffer (pH 7) to stand for 48 h, followed by centrifugation for 25 min at 14000 rpm to get rid of unbound oligonucleotide. Following removal of the supernatant, the red precipitate was resuspended to 5 ml of 0.1 M NaCl, 10 mM phosphate buffer (pH 7), recentrifuged for 25 min at 14000 rpm, removed the supernatant, redispersed in 1 ml of a $2 \times$ PBS stock solution, stored at 4°C , and diluted with equivalent MicroHyb buffer when used.

Surface treatment of polypropylene slices

The PP slices were cleaned in an acetone solution under supersonic waves, rinsed with water and dried under a vacuum. In order to link the amino functional group ($-\text{NH}_2$) on the surface of the PP slices, a plasma treatment was performed in a plasma producer with 100 W of discharge power, 2 Pa of vacuum, and a 2-min treating time. The gas flows of nitrogen and hydrogen were 50 ml/min and 100 ml/min, respectively. The treated slices were dipped in a 5% glutaraldehyde/10 mM PB solution for 2 h, and rinsed with 10 mM PB buffer and water. After being dried, the slices were immersed in a 10% hexamethylene diamine solution for 2 h at room temperature, washed thoroughly with water and dried prior to use.

In situ synthesis of DNA probes

DNA probes bound to grafted PP slices were *in situ* synthesized with an Expedite 8909 DNA synthesizer. The probe sequence was P2: 5'-TCA GTG CAT CGA TGT C-3'. The slice was deprotected in an ethanolamine/ethanol (1:1) solution at 76°C for 75 min, and washed in H_2O .

Hybridization of the sandwich assay format

In one set of experiments, a solution of the complementary target, T0 (based on the cardiac muscle blocking sequence) in

Fig. 1 TEM image of 13 nm Au nanoparticles.

MicroHyb buffer, was hybridized to an *in situ* synthesized oligonucleotide array for 4 h at 26°C , washed with a $2 \times$ SSC/0.1% SDS solution for 10 min, then with a $0.1 \times$ SSC/0.1% SDS solution for 10 min. Next, a solution of oligonucleotide-functionalized gold nanoparticles was hybridized to the array for 4 h at 26°C , washed with $2 \times$ SSC/0.1% SDS and $0.1 \times$ SSC/0.1% SDS solutions respectively, and then washed with $2 \times$ PBN [0.3 M NaNO_3 and $10 \text{ mM Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ buffer (pH 7)]. The hybridized PP slice was submerged in a Silver Enhancer Solution (solutions of 0.5 g $\text{AgNO}_3/2 \text{ ml H}_2\text{O}$, 1.7 g hydroquinone/30 ml H_2O and 2.55 g citric acid/2.35 g trisodium citrate/10 ml H_2O , mixed simultaneously) for 8 min at room temperature, and then washed with water. In another set of experiments, solutions of mismatched targets (T1, T2, T3) were hybridized to the synthesized oligonucleotide array, respectively. The procedure was entirely the same as that of a complementary target, T0.

Results and Discussion

Sensitivity of hybridization system

As a comparison, Figs. 2 and 3 showed the hybridization signals of *in situ* synthesized and spotted oligonucleotide-functionalized polypropylene slices that were exposed to various concentrations of oligonucleotide targets, labeled with a nano-gold probe and immersed in a silver amplification solution, respectively. The grayscale values of the hybridization signals are shown in Fig. 4.

The target DNA at concentrations as low as 100 fM was effectively distinguished from the background as the oligonucleotide (P1) was *in situ* synthesized onto the polypropylene slice surface, and the distinguishable concentration was about 100 pM with spotting oligonucleotide (P1). The sensitivity of the *in situ* synthesis system was 3 orders of magnitude higher than that of the spotting system, and the signals of the former were about 2-times stronger than that of the latter under the same target DNA concentration. This may be attributed to that *in situ* synthesized oligonucleotides were more uniform and more stably bound to the substrate surface than the spotted ones, thus presenting a better hybridization circumstance and a higher hybridization efficiency.

Specificity of the hybridization system

The signals of different target DNA sequences with the same concentration of 100 fM are shown in Fig. 5. T0, T1, T2 and T3

Fig. 2 Images of *in situ* synthesized oligonucleotide hybridization system; the concentrations of the complementary target DNA (T0) varied as indicated.

Fig. 3 Images of the spotted oligonucleotide hybridization system; the concentrations of the complementary target DNA (T0) varied as indicated. The controlled conditions were the same as for the *in situ* synthesis system.

Fig. 4 Grayscale of *in situ* synthesized and spotted probe (P1)-target (T0)-nanoparticle probe (P2)-silver amplified signals, obtained from histogram averages in Adobe Photoshop 7.0. 1, *in situ* synthesized probe; 2, spotted probe.

represented a perfect match as well as 1-base, 2-base and 3-base mismatch sequences, respectively. The complementary and mismatched target DNA could be distinguished from the background baseline. The ratio of the background-subtracted gray-scale values for T0:T1:T2:T3 is 22:16:9:4.

Conclusions

In summary, we demonstrated that this new gene-detection system, which employs a plasma treatment on polypropylene slices and a DNA *in situ* synthesis technique based on colorimetric detection, has a sensitivity as low as 100 fM, and can clearly distinguish mismatch sequences at this target

Fig. 5 Images of a different target DNA hybridization system at the same concentration of 100 fM.

concentration. The ratio of the background-subtracted gray-scale values for a perfect match, single-base mismatch, 2-base mismatch and 3-base mismatch is 22:16:9:4. The sensitivity of *in situ* synthesis system was 3 orders of magnitude higher than that of the spotting system, and the signals of the former were about 2-times stronger than that of the latter under the same target DNA concentration. The dominant advantage is the simple plasma treatment used to graft amino groups onto the polypropylene substrate surface, compared with the complicated surface treatment in other gene-detective methods. The excellent chemical and mechanical properties, ready availability of polypropylene slices, no need for optical setups and the high sensitivity of gold label silver stain detective method make this detective system both novel and attractive.

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