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FABRICATION OF DNA MONOLAYERS ON GOLD SUBSTRATES AND GUIDING OF DNA WITH ELECTRIC FIELD

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Abstract. We report electrically controlled selective coating of gold electrodes with mixed monolayers of oligonucleotides and alkanethiol passivation molecules. Gold nanoparticles are used as labels for visualization and voltage between the electrodes is applied for guiding the oligonucleotides. We discuss the efficiency of the guiding and monolayer preparation procedure.

1. INTRODUCTION

There have been many suggestions of using DNA (deoxyribonucleic acid) as a molecular wire in the electrical circuits, where the self-assembly properties of DNA molecule could be exploited. Electrical properties of DNA molecule have been studied widely, but the experimental results seem to be controversial [1,2,3]. Conducting behaviour of the double helical DNA has been observed for instance in [2] whereas a single-stranded DNA (ssDNA) molecule is claimed to be an insulator.

Possible electrical conductivity of double helix structure can be used in sequence-selective recognition of nuclear acid sequences. Our goal is to build a nanoscale sensor which could electrically monitor the forming of DNA double helix structure between two separated gold electrodes. By measuring the current through the helix we should be able to get information about the completeness of the formed structure. This kind of method makes it possible to perform fast and simple analysis of DNA sequences.

The specific recognition of DNA sequence is based on synthetic oligonucleotides, which are used as probes which are linked to gold surface via thiol-modifier groups. These probes attach to target DNA strands by hybridization reaction forming the double helix structure between gold electrodes. This could be observed by measuring the conductivity between the electrodes. Forming of the perfect double helix structure demands the base sequences in the single DNA strands to be perfectly complementary. Possible mutations in the double helix structure should decrease critically the conductivity of DNA. Observing of mutation announces that examinated DNA sequence is not identical with synthetic probes. Using present thin layer deposition techniques one can achieve very good vertical resolution. The deposited layer thickness can be controlled very accurately. Our main idea is to exploit thin film deposition techniques to fabricate a nanoscale sensor containing multilayer structures of thin metal and insulator films.

Since DNA-based pharmacological methods are becoming popular, DNA detection sensor has obvious applications in biological and medical analysis. The most common type of genetic variation is called a single-nucleotide polymorphism (SNP). These variations are associated with diversity in the population, individuality, susceptibility to diseases, and individual response to medicine. Recently, it has been suggested that SNPs can be used for homo-

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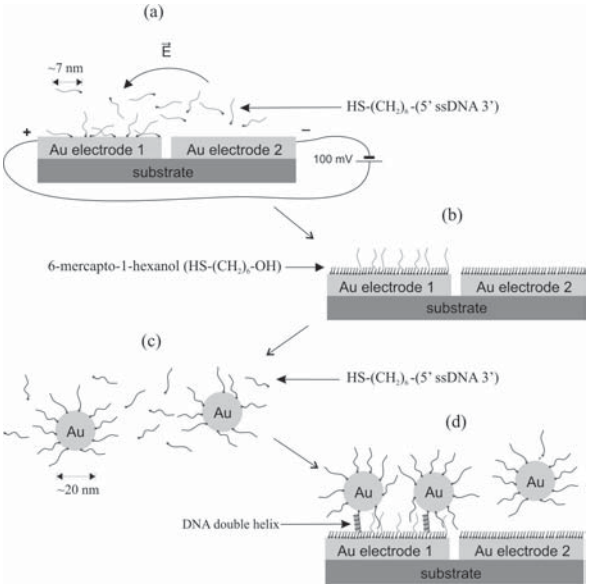

Fig. 1. Guiding oligonucleotides with electric field, DNA linked nanoparticles and mixed monolayer. (a) Negatively charged synthetic oligonucleotides are driven to contact with voltage positive electrode. Oligonucleotides are linked to the gold surface with thiol groups (Au-S bonding). (b) The gold surface is passivated with 6-mercapto-1-hexanol which attach to the surface with thiol group. (c) The other synthetic oligonucleotides are linked to 20 nm diameter Au nanoparticles. These oligonucleotides are complementary to the ones on the gold surface. (d) Nanoparticles attach to the gold surface with double helix structure formed by hybridisation of two complementary oligonucleotides. Unbound nanoparticles are washed away. Attached nanoparticles can be observed with electron microscopy.

geneity testing and pharmacogenetic studies and to identify and map common diseases such as high blood pressure, diabetes, and heart disease [4]. The heat-resistant structure of the sensor makes it possible to use it also in PCR-based applications. Furthermore, this sensor can be used for studying the conductivity of DNA giving essential information related to its potential use as a component of future electronics.

In this paper we report the first steps towards the realization of our proposed DNA sensor: electrically controlled selective coating of the gold electrodes with mixed monolayers of the probe oligonucleotides and alkanethiol passivation molecules. Gold nanoparticles are used as labels. Characterization of immobilized DNA has been done also by e.g. hydrozyl radical footprinting [5], fluorescence energy transfer [6], surface plasmon resonance [7], a combination of X-ray photoelectron spectroscopy and radiolabaling experiments [8] and neutron reflectivity [9]. Labeling with gold nanoparticles has been studied in [10]. In addition to techniques used in [10], we have used electric field as a driving force in the ssDNA assembly. The experimental results show the expected qualitative behaviour. We identify, however, several causes for the observed non-ideal behaviour.

2. VISUALIZING OF DNA MONOLAYERS WITH GOLD NANOPARTICLES

Gold electrodes of the DNA sensor must be coated with mixed monolayers which are formed of two components. The first component is thiol-modified oligonucleotide probes which hybridize with the target DNA. The second is 6-mercapto-1-hexanol which not only passivates the surface, preventing non-specific adsorption of DNA from solution, but also displaces non-specifically adsorbed HS-ssDNA (those molecules that interact with the surface through some functionality other than the thiol-group). In our recent experiments we have been studying the fabrication of these mixed monolayers.

We used two reverse-complementary 5'-thiol-modified synthetic oligonucleotides (5'-HS-ssDNA-3' from Synthegen), gold surface and self-made gold nanoparticles in our experiments. One of the synthetic oligonucleotides was used as probe DNA (5'-HS-$(CH_2)_6$-GAAGA-TGTTG-ATGCC-GACCC-3') and the other one was considered as a target DNA (5'-HS-$(CH_2)_6$-GCCAG-AAAGT-GCTCG-CTGAC-3'). Probe oligonucleotides are attached to gold surface while the target oligonucleotides are attached to the gold nanoparticles made in our laboratory (Fig. 1). These complementary oligonucleotides form the double helix structure which link the nanoparticles to the surface. Nanoparticles are then observed using scanning electron microscopy (SEM).

3. GUIDING OLIGONUCLEOTIDE PROBES WITH ELECTRIC FIELD

The gold surface was divided into two separate electrodes and a small voltage difference is set between those. We used voltages from 10 mV to 100 mV. In this way the oligonucleotide probes are driven with electric field in contact with positively charged electrode because the DNA backbone is negatively charged when kept in aqueous solution. When the

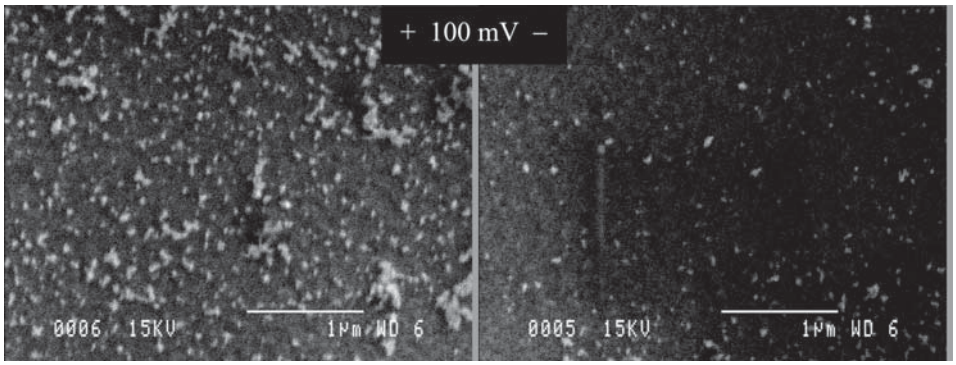

Fig. 2. Target oligonucleotide covered gold nanoparticles on the probe oligonucleotide modified gold electrodes. The fabrication was done in three incubation steps: (1) 10 nM thiol-modified oligonucleotides for 13 h when 100 mV voltage is applied between electrodes (positive electrode on the left), (2) 1 mM 1-mercapto-6-hexanol for 2 h and (3) 6 nM gold nanoparticles (20 nm colloidal gold) with 100 nM thiol-modified reverse complementary oligonucleotides in 10 mM NaCl.

probes are in contact with the gold surface, Au-S bonds can be formed. Detailed process steps are presented in Fig. 1.

We found that the expected behaviour presented in Fig. 1 is very sensitive to the process parameters (e.g. solution concentrations, incubation times and temperatures). However, we observed a successful process in several runs. For instance for the case given in Fig. 2 visualizes by SEM images that considerably more gold nanoparticles have been attached to the positive electrode, as expected.

4. NON-SPECIFIC BINDING OF OLIGONUCLEOTIDES

Although results such as presented in Fig. 2 indicate that the process of Fig. 1 works to some extent, it clearly has to be improved and optimised to be useful for the detector fabrication application. Several (in this context) undesired effects may contribute, therefore the following experiments were performed to identify their influence.

1. Experiments were done with pure gold surface and target oligonucleotide coated gold nanoparticles, without the probes on the surface (Fig. 3). Oligonucleotide coated nanoparticles were prepared from 6 nM oligonucleotides and 600 nM (or 60 nM) target oligonucleotides. We observed that more particles were attached to the surface when more target oligonucleotides were used in the coating procedure. These results indicate that nanoparticles can attach to the surface also with other groups than synthetic thiol- modifiers. This non-specific binding be-

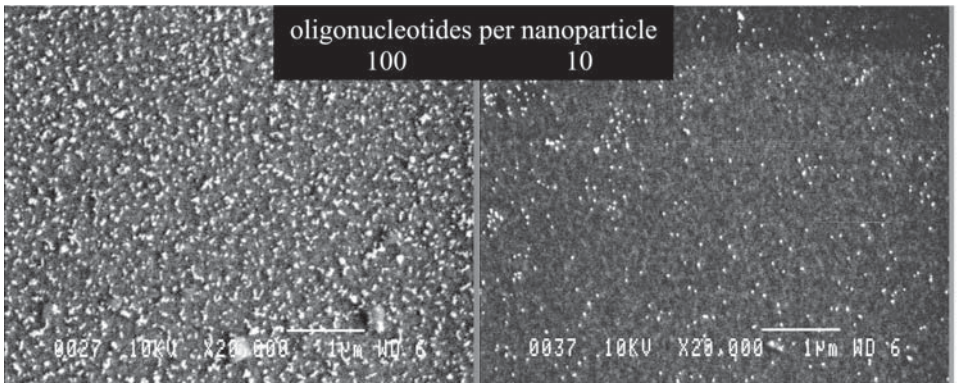

Fig. 3. Oligonucleotide coated nanoparticles on the pure gold surface. Surface is incubated for 2,5 hours with 6 nM nanoparticles in 20 mM NaCl solution. There are about 100 oligonucleotides per nanoparticle in the left picture and about 10 nucleotides in the right. Surface was cleaned with piranha before incubation.

tween oligonucleotides and the pure gold surface was observed also in [5].

2. Experiments with double helix coated nanoparticles where also made. Target oligonucleotide coated nanoparticles were mixed with probe oligonucleotides so that the complementary sequences could hybridize and were then exposed to the pure gold surface. Helix coated nanoparticles were prepared from target oligonucleotide coated nanoparticles by adding 1000 nM (or 100 nM) probe oligonucleotides in 20 mM NaCl. More aggregates were observed with oligonucleotide coated particles than helix coated particles, which refers to aggregation of particles through the non-specific binding of oligonucleotides. We also observed that helix coated nanoparticles did not attach to the surface as well as the target oligonucleotide coated nanoparticles: one may propose that nanoparticles attach to the surface also via bases.

5. DISCUSSION

We have performed experiments on attachment of oligonucleotides on gold surface combined with voltage guiding. Voltage guiding worked in principle, but the electrode configuration and voltage ranges have to be optimised. Oligonucleotides were observed to attach to the gold surface not only with thiol-groups, but also with non-specific binding. More detailed quantitative analysis will be needed to conform these preliminary results. Detailed understanding of the attachment to metals and the voltage guiding of DNA will be useful for many detector applications and to enable fabrication of structures for fundamental studies of the electrical properties of DNA.

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