Direct Detection of Long, Periodic, ssDNA nanostructures Assembled on CMOS Transistor Arrays

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Abstract—DNA is a wonderful material for the construction of nanostructures for a variety of applications. This paper describes a novel platform integrating single-stranded DNA (ssDNA) nanotemplates with CMOS-compatible, field-effect sensors. The field-effect sensor was based on oxide-semiconductor field-effect transistors (OSFETs), which were monolithically-integrated with signal-processing circuits to enhance the signal-to-noise ratio, and to detect the construction of ssDNA nanotemplate from complex DNA-protein interactions. The ssDNA nanotemplate on the transistor arrays was prepared by isothermal rolling circle amplification (RCA) through DNA aptamer-protein recognition and self-assembly strategy. The growth of the DNA nanostructure was monitored in situ, real-time and label-free on OSFET.

I. INTRODUCTION

DNA nowadays is more than just a carrier of genetic blueprint for life. It is used as a building block to construct novel nanomaterials for nanodevices [1]. Long, periodic ssDNA becomes a promising nanotemplate for the construction of 2D nanostructure by the self-organization strategy. There are accumulating studies in the construction of DNA nanostructure. The atomic force microscope was adopted to visualize DNA nanostructure directly without the quantitative analysis Utilizing gel electrophoresis technology further allows the amount of DNA to be measured quantitatively, but damaging the 3D conformation of the DNA in the purification process. Here we provide a novel, label-free platform able to construct and measure in situ the long, functionalized, periodic ssDNA nanotemplate.

II. EXPERIMENTAL

A. The fabrication of sensor structure

Fig.1 shows the layout of a multi-finger OSFET compatible with standard CMOS process [2]. The dashed circle indicates the active (sensing) region. In order to enhance the sensitivity, all materials above gate-oxide in the active
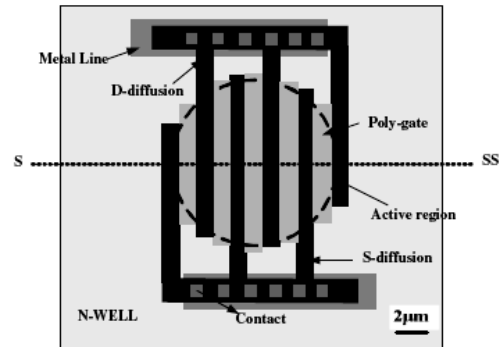

Figure 1. The layout of a multi-finger OSFET

region are removed to form an open-gate, field-effect transistor structure. Potential changes above the gate-oxide surface are transformed into changes in the channel current (flowing between the S- and D- diffusions) directly. Moreover, the multi-finger structure is employed to maximize the transconductance within a specific sensing area.

Fig.2 shows the post-CMOS processes for fabricating the OSFET at the die level. P-channel MOSFETs were first fabricated with the standard TSMC 0.35μm CMOS process, in which metal layers were used to define the active region. The metal layers were then removed by wet etching with "piranha" at 85°C to expose the poly-gates of the transistors. Afterwards, reactive-ion etching (RIE) was applied for five minutes to remove the thin silicide layer above the poly-gates. The poly-gates was then wet-etched with KOH: DI water = 2:1 at 80°C for 20 seconds to expose the gate-oxode. Finally, with a fraction of silicon wafer functioning as shadow mask, the passivation over bonding pads was exposed by the RIE. Fig.3 shows the photos of the P-channel OSFET sensors before and after the post-CMOS processes.

After the post-CMOS processes, the chips were wire-bonded to a printed-circuit board (PCB), to which a glass O-ring was attached to form a bath, as shown in Figure 4. The entire chip surface except for the OSFET area was then coated with industrial epoxy (WK-8126H, WinKing) to prevent short circuits introduced by solutions in the bath
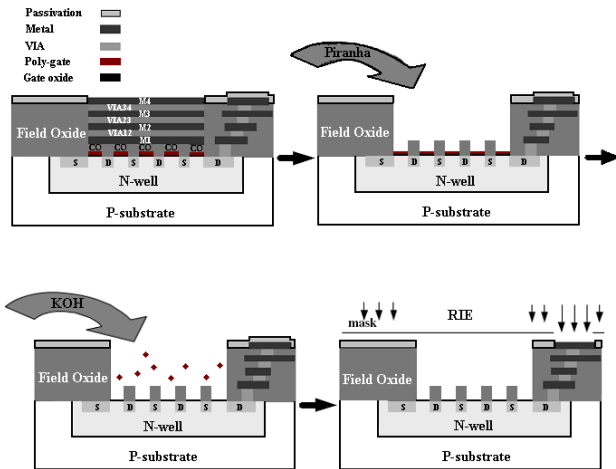
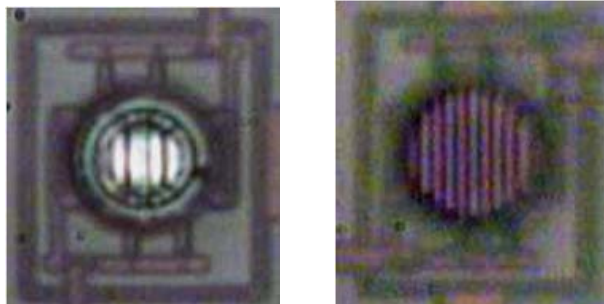Figure 2 The post-CMOS process flow for the OSFET fabrication

Figure 3. Microphotographs of (a) original P-MOS transistor (b) P-channel OSFET after the post-CMOS process
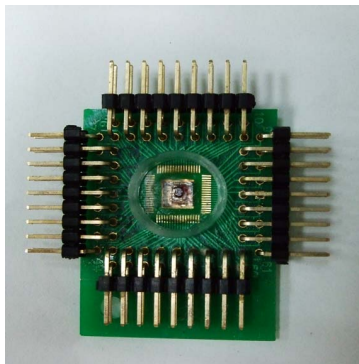

Figure 4. Photograph of a packaged multifinger OSFET fabricated by the standard CMOS process for rolling circle amplification (RCA)

B. The measurement setup

The Keithley 2602 series SourceMeter was used to bias and measure the OSFET sensors, with the source meter configured through the software "Lab Tracer", as shown in Fig(5). As the gate material of the OSFETs has been replaced by the solution in the glass O-ring, an Ag/AgCl electrode was employed to provide the DC bias for the solution. With the drain (D) and the solution voltages kept at 0V, the channel current of the OSFET was measured when the source voltage
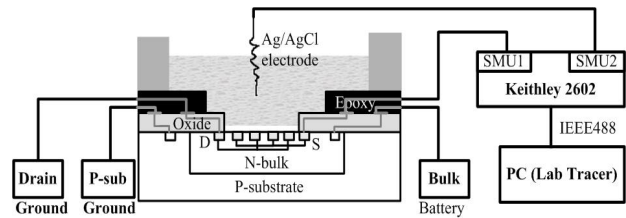

Figure 5. The setup for measuring the I-V curves of the OSFETs in response to different DNA interactions

(S) was swept from 0 V to 3 V with a step of 50mV. The current-voltage relationships at different stages of DNA synthesization were measured and transmitted to the PC via the IEEE488 cable.

C. The method to construct the DNA nanostructure

The ssDNA nanotemplate in this study were synthesized on the gate-oxide surface of the OSFET by using the self-assembly monolayer (SAM) as the covalent linker between the DNA and the silicon oxide surface. There are three key technologies in the system, including: 1. conformation-switched aptamer induced by platelet-derived growth factor (PDGF), a tumor marker; 2. ssDNA nanotemplates synthesized by the RCA; 3. molecular sensing of the ssDNA nanotemplates by OSFET (Fig.6). All DNA strands used were commercially synthesized from (Genedragon, Taiwan) and purified by denaturing gel electrophoresis. The DNA sequence was designed and modified from the sequences previously-reported in [3].

The SAM membrane of APTES was formed on silicon-based substrate by silanization. The cover glass substrate was first washed by ethanol solution to remove contaminants, incubated with 2.0 % APTES ethanol solution for 30 min.s, and heated at 120   for 10 min.s to remove excess ethanol. Secondly, the substrate was treated with solution containing 2.5% glutaraldehyde and 4 mM sodium cyanoborohydride for 1.5 hr followed by water wash. Finally, the 500 nM 5'-aminomodifed primer was coupled to the glass substrate at 4   overnight PDGF-BB. The human recombinant homodimer was purchased from Upstate, USA and reconstituted in 4 mM HCL with 2 mg/ml bovine serum albumin (BSA) as suggested by the supplier. 3-Aminopropyl triethoxysilane (APTES), BSA, Tween 20, sodium cyanoborohydride (NaBH3CN), phosphate buffer saline (PBS), and ethanolamine were purchased from Sigma–Aldrich (USA). 25% glutaraldehyde in aqueous solution was purchased from Fluka (USA). T4 DNA ligase, Phi29 DNA polymerase were purchased from Epicentre (USA). T4 Gene 32 protein was purchased from Ambion (USA). All reagent solutions were prepared and steam sterilization with DIW (resistance of water was 18.2 MΩcm) from an ultra-pure water system (Sartorius, Germany).

Aptamer was an artificial synthesized ssDNA molecule with a three-dimensional structure. The aptamer was able to recognize the target protein, PDGF, specifically and the recognition process transformed the DNA aptamer into a circular form. 5 nM PDGF was incubated with the 40 nM PDGF aptamer in a nucleic acid ligation reaction, and the

ligation reaction was terminated by heating at 95 ℃ for 5 min.s. The circularized ssDNA were then added into the RCA reaction chamber on the OSFET chip and triggered the RCA reaction.

III. RESULTS AND DISCUSSION

An *in situ* construction of the ssDNA nanotemplate on the surface of an OSFET is illustrated in Fig.6. The technologies of aptameric recognition and rolling circle amplification were merged to construct the long, periodic ssDNA as a nanotemplate. Platelet derived growth factors (PDGF) was recognized by the DNA aptamer and induced the circularization of aptamer (Fig. 7a). The circularized aptamer was added into the solution above the OSFET sensors, and the aptamer was complementary to the RCA primer immobilized on the OSFET surface. Subsequently, the ssDNA nanotemplate was polymerized by the RCA reaction with T4 gene32 as ssDNA binding protein. Phi29 DNA polymerase acted both on the DNA polymerization and ssDNA displacement, which was a unique property in the DNA polymerse (Fig. 7b).

The entire process of the *in situ* replication of the DNA nanotemplate was successfully detected by the OSFET chip. As shown by Fig.8, a left shift of the Id-Vsg curve of the OSFET was observed when ssDNA primers were immobilized on the SAM layer of the transistor. The left shift indicated that more negative charges accumulated on the surface of the OSFET because the current of a p-channel transistor increased with its Vsg. This result agrees with the fact that the ssDNA primers were negatively-charged, demonstrating the capability of the OSFET to detect the immobilization of the DNA primer. Moreover, as the ssDNA nanotemplates were constructed by the RCA process, the Id-Vsg curve shifted further left as shown in Fig.9. This change was simply attributed to more negative charges introduced by the elongated ssDNA nanotemplates.

IV. CONCLUSION

By immobilizing DNA primers on the CMOS-compatible OSFET sensors, the feasibility of monitoring the synthesization of DNA nanotemplates *in situ* is demonstrated. This approach provides the advantage of detecting the interactions between the DNA and the (PDGF) proteins in real-time without damaging the DNA strands. The CMOS-compatibility allows the sensors to be further integrated monolithically with amplifying circuits, so as to sense subtle interactions between nanoscale biomolecules. Moreover, the label-free detection of the RCA process is first demonstrated. As the RCA process allows DNA strands to be amplified at room temperature, the sensory drifts of OSFETs induced by temperature changes are directly avoided. Therefore, with different DNA nanostructures, the proposed platform will be useful for many molecule biology researches, as well as for disease diagnoses.
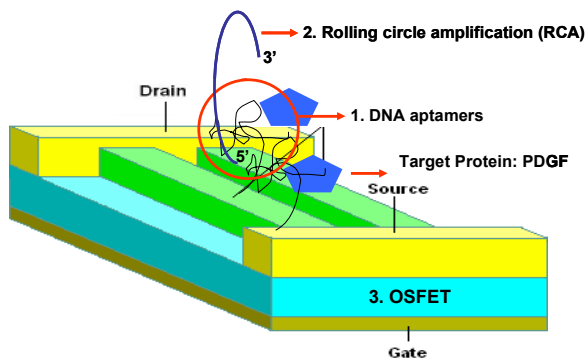

Figure 6. Schematic diagram of a OSFET biosensor integrated with the ssDNA nanostructure.
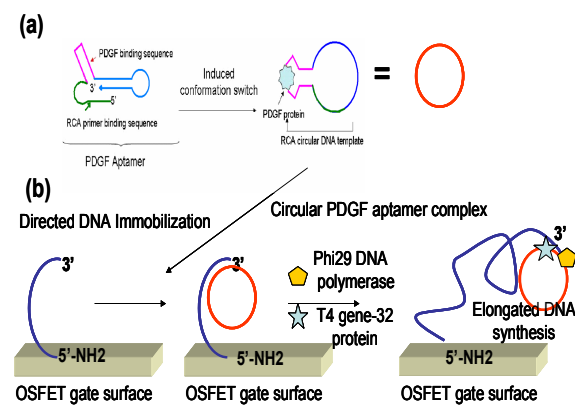

Figure 7. Construction of ssDNA nanostructure. (a) Aptamer-protein recognition to form the circular RCA template (b) The progress of long, periodic ssDNA nanostructure on the OSFET surface.
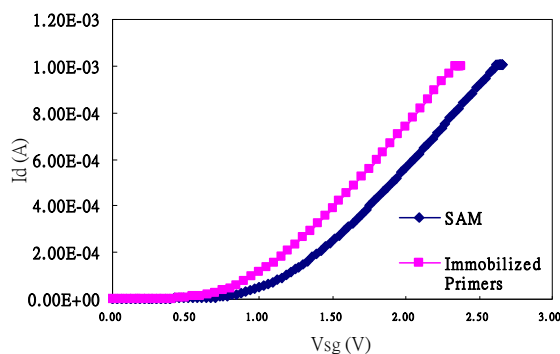

Figure 8. Electrical responses of an OSFET sensor before and after surface functionalization. Id-Vsg curves obtained from the SAM layer formed on the OSFET silicon surface (denoted as SAM), and from 500 nM ssDNA primers (denoted as Immobilized primers) immobilized on the SAM surface of OSFET respectively.
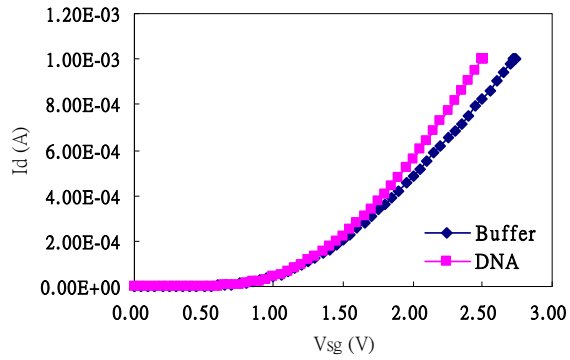
Figure 9. The Id-Vsg curves of the OSFET measured before and after long, periodic functionalized ssDNA nanostructures were self-assembled through the RCA at room temperature.

V.   ACKNOWLEDGEMENT

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