DNA detection on transistor arrays following mutation-specific enzymatic amplification

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An integrated array of silicon field-effect transistor structures is used for electronic detection of label-free DNA. Measurements of the dc current–voltage characteristics of the transistors gives us access to reproducible detection of single- and double-stranded DNA, locally adsorbed on the surface of the device. We combine this approach with allele-specific polymerase chain reaction, to test for the 35delG mutation, a frequent mutation related to prelingual nonsyndromic deafness. © *2004 American Institute of Physics.* [DOI: 10.1063/1.1650907]

Recent years have seen increasing research activity devoted to electronic measurements on systems issued from cellular and molecular biology. In this field, an important class of approaches is based on semiconductor field-effect transistor (FET) structures, to detect a change in potential of a solid/liquid interface. At a cellular level for instance, electronic detection of neuronal activity has been achieved by measuring current-induced potential differences with spatial resolution using integrated FET arrays.[1] In the present work, similar FET arrays are used for biomolecule detection. We find that immobilization of charged molecules on the SiO_2 surface of these arrays modifies the conductivity of the bidimensional gas of charge carriers located underneath. This FE transduction principle was initially introduced by Bergveld.[2] The electronic detection of biomolecules binding to individual FETs has been studied by several groups,[3–6] while the differential measurements used here rely on the use of arrays rather than individual FETs.

The electronic detection of biomolecules by their intrinsic charge is neither specific to DNA (most biomolecules are charged in solution) nor specific to a particular base sequence. Here, specific detection of a point mutation is achieved by combining the electronic measurement with an allele-specific polymerase chain reaction (AS-PCR).[7] We apply this approach to the detection of the 35delG mutation. This mutation occurs in the connexin (CX26) gene on chromosome 13 and is one of the most frequent known mutations in the human genome. It is related to prelingual nonsyndromic deafness, a common hereditary sensory defect.[8]

We use integrated silicon *p*-channel FET arrays with 62 or 96 FETs, linearly arranged with a period of 20, 30, or 40 μm. The individual sensing areas, 24, 36, or 40 μm^2 in size, are covered by a 10-nm-thick SiO_2 layer. These semiconductor devices have been provided by the Max-Planck-Institute for Membrane and Neurophysics, Germany. Their fabrication has been described.[9,10]

During the electronic measurements, the sample surface is immersed in a KCl electrolyte solution. Two dc voltages, U_S and U_{SD}, are generated by a multifunction I/O board, lowpass filtered, and are applied to the FET array with an analog amplifier circuit. U_S is applied between the common source and an Ag/AgCl electrode introduced in the electrolyte. U_{SD} is applied between source and drain. We measure the drain current I_D for all transistors as a function of U_S and U_{SD}, by multiplexing with a computer controlled switch unit. By numerical interpolation of the $I_D(U_S, U_{SD})$ characteristics, we derive the voltage U_S that corresponds to a given (I_D, U_{SD}) working point.

Initially, the SiO_2 surface of the semiconductor devices are incubated 1–2 min in sulfochromic acid, rinsed with deionized water, incubated in a NaOH/ethanol solution, rinsed again and dried. Subsequently we incubate in a poly(L-lysine) dilution for 30 min (P8920 Sigma, 0.01% wt/vol in 0.1× PBS buffer at pH7), rinse with H_2O, and dry with air.

With a custom microspotting setup that allows one to visualize the local deposition on the semiconductor surface, several spots containing DNA oligonucleotides are placed on the FET array. For the experiment presented in Fig. 1, a commercial stealth microspotting pin (Telechem, SMP3B) has been used, which nominally delivers a liquid volume of 0.9 nl to a circular area of $\sim$100 μm in diameter. In practice, these values depend on the wetting properties of the surface. As shown in Fig. 1, the local deposition of DNA leads to sizeable negative shifts in U_S for the transistors that have been exposed to the molecular charge (each spot covers 6–7 FETs). The shifts in U_S depend barely on the $\{I_D, U_{SD}\}$ working point. In addition, comparing two batches of FET arrays exhibiting different lateral dimensions of the active surfaces and different current–voltage characteristics, the shifts in U_S are found to be the same within the sample-to-sample reproducibility. The shift in U_S thus represents an appropriate characterization of the electrical properties of the SiO_2/biopolymer/electrolyte interface.

After deposition with a spot diameter of $\sim$100 μm, we detect 20 mer oligonucleotides down to 10 μM in concentration. At lower concentrations, we are limited by the fact that drying of liquid buffers on the active surfaces can induce shifts in U_S of the order of 10 mV. To estimate the number of DNA bases adsorbed to the active surface of an individual FET, we assume that after deposition, the liquid droplet dries and defines a disk-shaped area of constant areal DNA den-

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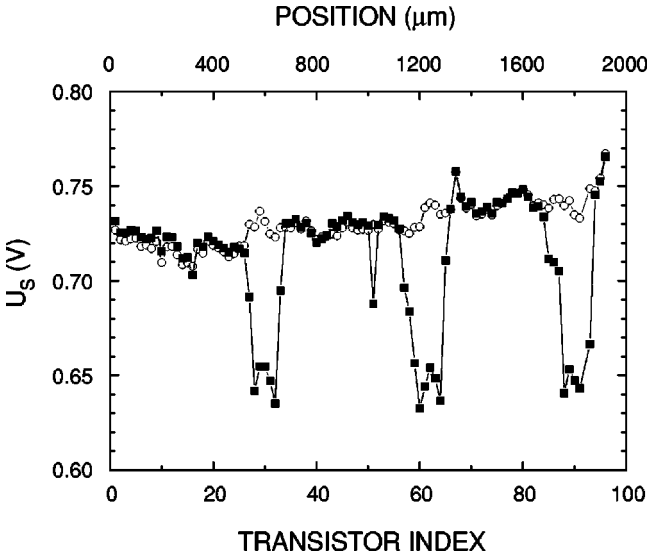

FIG. 1. Electronic detection of DNA oligonucleotides. The voltages U_S corresponding to a working point $I_D=100\ \mu$A and $U_{SD}=1$ V are plotted as a function of the transistor index (bottom scale) and position (top scale). The open circles represent a measurement performed after poly(L-lysine) incubation, the full squares a measurement performed after subsequent local deposition of DNA (three spots taken from the same solution, 20 mer oligonucleotides, 50 μM in H_2O). Measurements have been done at room temperature with a 0.01 mM KCl electrolyte. A linear array with 96 transistors, individual sensing areas of 40 μm^2, and a period of 20 μm has been used.

sity. With a concentration of 10 μM and a spot diameter of 100 μm, we thus get a surface density of 2×10^5 molecules/μm^2. With 24 μm^2 for the active area of an individual FET and 20 DNA bases per 20 mer oligonucleotide, this leads to a value of 10^8 as an estimation of the number of DNA bases per transistor needed for electronic detection in the present configuration.

In aqueous solutions at neutral *p*H, single- and double-stranded DNA molecules are negatively charged, while poly(L-lysine) has a positive charge. The shifts in U_S that we obtain upon adsorption of these two polymers are consistent with the fact that we use *p*-channel devices: for DNA, we observe $\Delta U_S<0$, while for poly(L-lysine), $\Delta U_S>0$ [difference between a measurement after poly(L-lysine) adsorption and a measurement after the NaOH/ethanol step]. The salt concentration of the KCl electrolyte used in the measurement influences the amplitude of the shifts. As expected for screening of the molecule charge by mobile ions, the measured amplitudes decrease with increasing salt. A typical result observed for oligonucleotide adsorption is: $|\Delta U_S|=80$ mV at $[KCl]=0.01$ mM, 40 mV at 1 mM and $|\Delta U_S|<10$ mV for $[KCl]>10$ mM.

In the following, we show how a point-mutation can be detected in DNA by AS-PCR followed by electronic detection of the double-stranded PCR product. The PCR and the electronic detection are separated by a purification that retains PCR product and genomic DNA, but eliminates nucleotides, primers, proteins, and salt. As very little substrate DNA is used, the PCR product represents the only species susceptible to induce a significant signal in the electronic detection. Finally, comparative analysis of two different samples on the FET array provides reproducible mutation detection, the overall specificity is simply that of the AS-PCR.

In a first step, a 940 base-pair (bp) fragment of the human CX-26 gene (accession code M86849, chromosome 13q11-12) is amplified by regular (not AS) PCR from genomic DNA extracted from different patients. Here, we use primers and cycling conditions as in[8,11] and a DNA polymerase with improved sequence fidelity (Pwo polymerase, Roche Diagnostics) in a 1.5 mM $MgSO_4$ PCR buffer. The PCR products of this preamplification step are purified on spin columns (QIAGEN) and, after dilution (10 000 fold), serve as template DNA in the subsequent AS reaction.

To detect the 35delG mutation in the CX26 gene, we have set up AS-PCR, with cycling conditions and primer sequences from Ref. 12. Two different mutation-specific forward primers and a common reverse primer are used to synthesize 197 bp PCR products. We perform two AS-PCRs on each DNA sample. One reaction involves the first specific primer (named MUT) and gives a product specifically if the mutation is present in the template. Another one is set up with the second specific primer (named WT) and gives a product specifically if the mutation is absent. The two reactions allow one to determine whether a sample is homozygous normal (product present only for the second reaction), heterozygous (product present for both reactions), or homozygous for the mutation (product present only for the first reaction). For a 50 μl reaction in standard PCR buffer, we use 1 μl of DNA from the preamplification step just described, primers at 30 pmol each, dNTPs at 100 μM each, and 1 μl TAQ polymerase (1U/μl, Roche Diagnostics). These PCR products are purified twice on spin columns and eluted with 10 mM Tris-Cl buffer at pH 8.5.

The example presented in Fig. 2 is based on two different patients, p1 and p2. The genomic DNA used here has been screened for mutations in the CX26 gene by sequencing.[11] Patient p1 is homozygous normal for 35delG, while patient p2 is heterozygous. Performing the two different AS-PCRs (based on either primer WT or primer MUT) on the genomic DNA of the two patients (p1 or p2), we get four products, called WTp1, WTp2, MUTp1, and MUTp2.

We detect the double-stranded products of these four reactions in parallel, using a semiconductor structure with two linear arrays of 31 FETs. The sample has been coated with poly(L-lysine) for DNA immobilization and the FET arrays have been measured for reference, as described earlier. For simplicity, we used here a micropipet to deposit 0.15 μl of each product on separate parts of the surface, as schematically shown in the top part of Fig. 2. We incubate 15 min without drying, rinse with water, and subsequently measure the current–voltage characteristics of the transistors. In the bottom part of Fig. 2, the shift ΔU_S between the measurement after deposition and the reference measurement is presented as a function of the transistor index. Sizeable downshifts in U_S are observed for WTp2, WTp1, and MUTp2. In contrast, for MUTp1, the shifts are comparable to the ones observed for the reference regions that received no DNA. The electronic measurement thus gives the result that, regarding 35delG, patient p1 is homozygous normal and patient p2 heterozygous. This agrees with the result obtained by direct sequencing. The concentration of the double-stranded

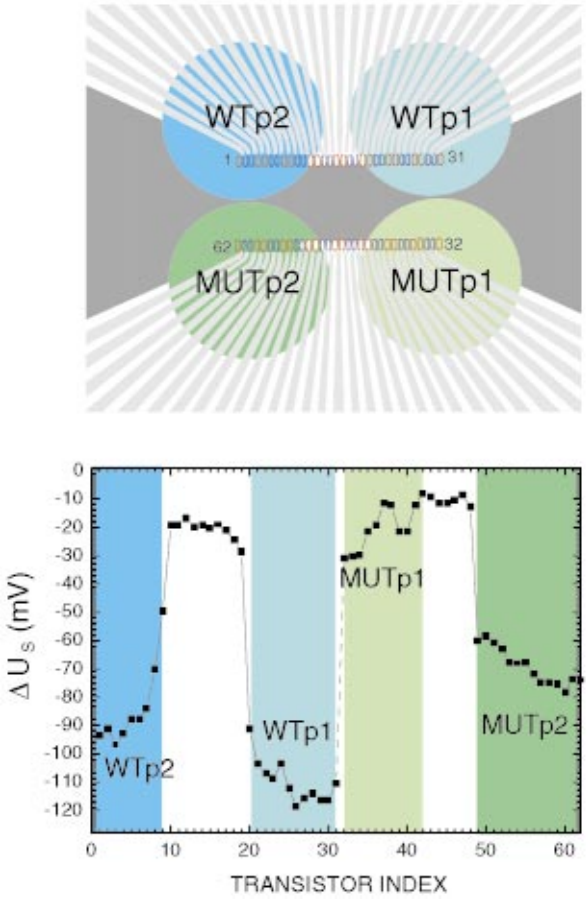

FIG. 2. (Color) Genotyping two patients (p1 and p2) with respect to the 35delG mutation. For each patient, two PCR runs are performed with AS primers WT and MUT. The four products are spotted to separate parts of the FET array (top) and electronic measurement of the surface-bound double-stranded PCR fragments is performed at a salt concentration of [KCl] $=0.01$ mM (bottom). The shifts in U_S plotted here as a function of the transistor index correspond to $I_D=100\ \mu$A and $U_{SD}=1$ V. The array consists of two parallel lines of 31 transistors separated by 0.5 mm. Within both lines, FETs with sensing areas of 36 μm^2 alternate with FETs of 24 μm^2, and the period is either 30 or 40 μm.

PCR product in tubes WTp2, WTp1, and MUTp2 is ~ 20 ng/μl, as determined by agarose gel electrophoresis. We thus used ~ 3 ng DNA per spot. If needed, this quantity could be reduced by the use of the microdeposition described earlier. The parallel measurement of four samples deposited on different parts of an integrated array illustrates the differential approach underlying this work. The comparison of different transistors in contact with a same electrolyte solution circumvents the difficulty that the current–voltage characteristics of an individual FET depends on *p*H and salt.

AS-PCR is one of several techniques for genotyping mutations and single nucleotide polymorphisms in DNA.[13,14] It has the advantage of relative low cost, but suffers from a somewhat limited flexibility. The AS primers have to overlap the polymorphic site, which can make difficult the design of a robust assay in problematic sequence contexts. The combination of AS-PCR with label-free detection on planar surfaces could be of particular interest in future integration with microfluidic DNA amplification in lab-on-a-chip technology.[15,16] Other approaches for label-free detection of DNA include mechanical cantilevers, quartz crystal microbalances, impedance spectroscopy, absorbance at 260 nm, and surface plasmon resonance. Up until now, label-free techniques have not been used in conjunction with AS-PCR.

In conclusion, we have shown electronic detection of label-free single- and double-stranded DNA by dc measurements on silicon FET arrays. Sequence specificity is obtained by AS-PCR.

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