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Comparison Between Electrochemical and Optoelectrochemical Impedance Measurements for Detection of DNA Hybridization

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Abstract

The principles of the electrochemical and optoelectrochemical impedance measurements on bare electrolyte/dielectric/semiconductor structures are described. The analysis of the experimental curves allows access to several indications concerning the electrical behavior of such structures. The application of these techniques to follow the electrical behavior of structures modified with two biological systems was investigated. The antibody/antigen recognition did not change the surface charge and, therefore, did not affect the impedance curves with respect to the applied potential. By contrast, the hybridization of two complementary DNA strands on the surface of the structure induced a variation of flat band potential of the semiconductor leading to a shift of impedance curves along the potential axis. This means that it is possible to detect directly the DNA hybridization without the use of labeled probes. The use of light allows the surface to be probed locally. In the future, the application of this technique for direct detection of hybridization on DNA chips should be possible.

Index Entries: DNA; hybridization; biosensor; electrochemical impedances; optoelectrochemical impedances.

Introduction

In the field of biosensors, affinity systems take a special place because of their properties of specific and selective recognition without consummation

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or transformation of species. The difficulties lie in the fact that interactions do not produce electronic transfers and no current is produced during the recognition. Currently, the detection of the biological recognition requires the use of labeled targets (enzymatic, radioactive, or fluorescence labels). In this article, we describe how to use the properties of a semiconductor for transducing the biological recognition into an electrical signal that is easy to measure. Particular structures were elaborated and studied with impedance measurements.

Electrochemical Impedance on Electrolyte/Dielectric/Semiconductor Structures

At the beginning, the studied structures consisted of a semiconductor (generally silicon) covered with a nonconductive layer made of silicon dioxide or silicon nitride. On the rear side, an ohmic contact allows polarization of the structure put in contact with an electrolytic solution. The insulating layer helps avoid some of the difficulties connected to eventual corrosion of the semiconductor material in contact with solution and to release from several faradic processes. Thus, the electrochemical properties of such electrolyte/dielectric/semiconductor (EDS) structures can be analyzed thanks to the electrochemical impedance measurements. Because the structure is a blocking electrode, no faradic process occurs. In this case, the electrical behavior of the structure is strongly dominated by both the capacity of the space charge layer in the semiconductor and the capacity of the dielectric layer. These capacities generally reach values in the range of 10^{-6}–10^{-7} F/cm^2 and correspond to a large part of the total impedance. The frequency spectral domain is limited at about 100 Hz for the low frequency because of the signal-to-noise ratio, and at 100 kHz in the high frequency owing to the instability of the potentiostatic setup. The electrical equivalent scheme is constituted of resistive and capacitance elements, whose values vary greatly with the applied potential to the structure. Thus, it is more convenient to perform impedance measurements at a fixed frequency for various potentials applied to the structure. For a given voltage, impedance measurement requires weak perturbation of the system around its thermodynamic equilibrium with a weak sine-modulated signal. A fast, homemade, three-electrode potentiostatic device monitors the voltage (V) applied to the working electrode referred to a reference electrode—saturated calomel electrode (SCE) or Ag/AgCl electrode. This potenstiostat is designed to add the modulated signal of small amplitude (v = 10 mV) in the frequency range of 10 Hz to 100 kHz delivered by a function generator to the applied potential. A lock-in amplifier measures alternatively the voltage $V + \nu$ and the current flowing through the structure. The impedance term is calculated by using Ohm's law with a complex term as follows *(1)*:

$$Z_p + iZ_q = (V_p + iV_q)/(I_p + iI_q)$$

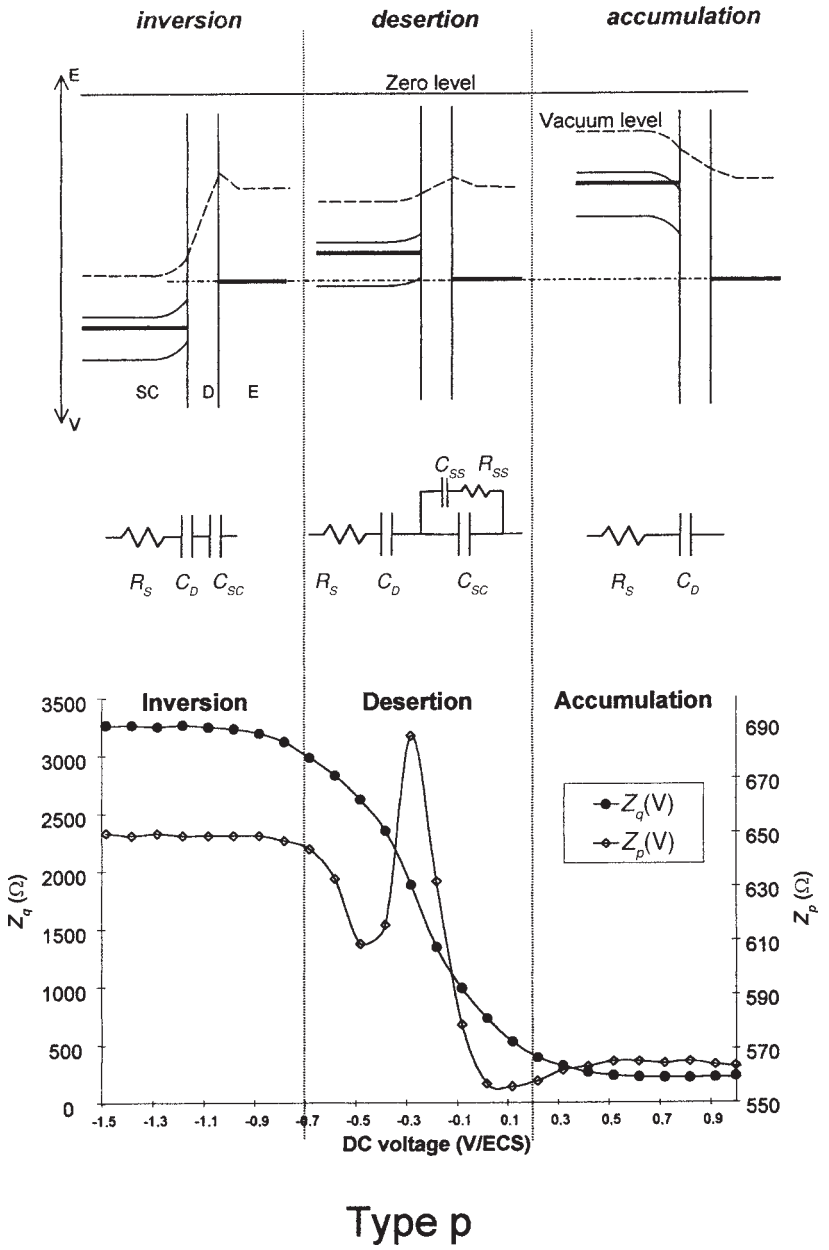

Fig. 1. Experimental impedance curves performed on pSi/SiO_2/electrolyte with associated equivalent circuits and energy diagrams. Z_p is the in-phase term and Z_q is the out-of-phase impedance.

Then,

$$Z_p = [(V_p I_p + V_q I_q)/(I_p^2 + I_q^2)] \text{ and } Z_q = [(V_q I_p - V_p I_q)/(I_p^2 + I_q^2)]$$

Figure 1 shows the impedance curves obtained on these EDS structures at a frequency value of 100 kHz. The use of high-frequency modula-

tion allows partial separation of the capacitance effects from the conductive ones. Thus, the out-of-phase impedance curve (Z_q) translates the different capacitance properties from both the space charge layer in the semiconductor (C_{sc}) and the dielectric (C_d) in relation to the applied potential. For instance, three polarization ranges corresponding to inversion (V <–0.7 V/SCE), depletion (–0.7 V/SCE < V < +0.2 V/SCE), and accumulation (V > + 0.2 V/SCE) situations can be distinguished from the (Z_q-V) curve obtained on p-type Si/SiO_2 structures in the electrolyte.

Figure 1 also gives the energetic diagram and the equivalent electrical scheme corresponding to three different potential ranges. In the strong inversion situation, the dielectric capacitance can be neglected in comparison with C_{sc}, and the total capacitance value is directly in relation with the doping level of the semiconductor. By contrast, in the accumulation situation, the semiconductor capacitance C_{sc} value is very high, and, therefore, the out-of-phase impedance is owing only to the dielectric capacitance. If, ε, the dielectric constant of the SiO_2 layer, is known, the thickness (d) of this layer can be deduced from the Z_q value by using the following equation *(2)*:

$$d = 2\pi F \varepsilon \varepsilon_0 S Z_q$$

in which S is the area of working electrode in contact with the solution; F is the frequency modulation; and ε_0 is the permittivity. For intermediate voltages, the electric field into the space charge layer and, consequently, the C_{sc} value vary strongly. This induces the decrease in out-of-phase impedance as can be observed on the experimental curve. During the voltage scan, polarization goes through a noteworthy value called flat band potential, V_{fb}. This potential corresponds to the required potential to cancel the band bending of the semiconductor occurring in the space charge layer. V_{fb} allows the determination of the energy level of the structure in relation to the solution.

The in-phase impedance noted Z_p results from the electrolyte impedance, bulk impedance, and superficial layer of the semiconductor. The Z_p vs voltage dependence is mainly owing to the charge carrier recombination either directly between the conductive and valence bands or via the surface states located at the semiconductor/dielectric interface. Thus, the peak appearing on the Z_p (V) curve was related to the surface states present in the band gap. The position and the amplitude of this peak characterize the energy level and the density of these surface states, respectively, as reported previously *(3)*. That is why this peak can differ from one electrode to another, depending on the Si/SiO_2 interface.

Electrochemical impedance measurements therefore give access to much information about the electrical behavior of the EDS structure. These data concern the dielectric (thickness, charge density), the semiconductor (p or n type, doping level), the quality of the dielectric/semiconductor contact (surface state density and their energy levels), and the dielectric/electrolyte interface (charge density).

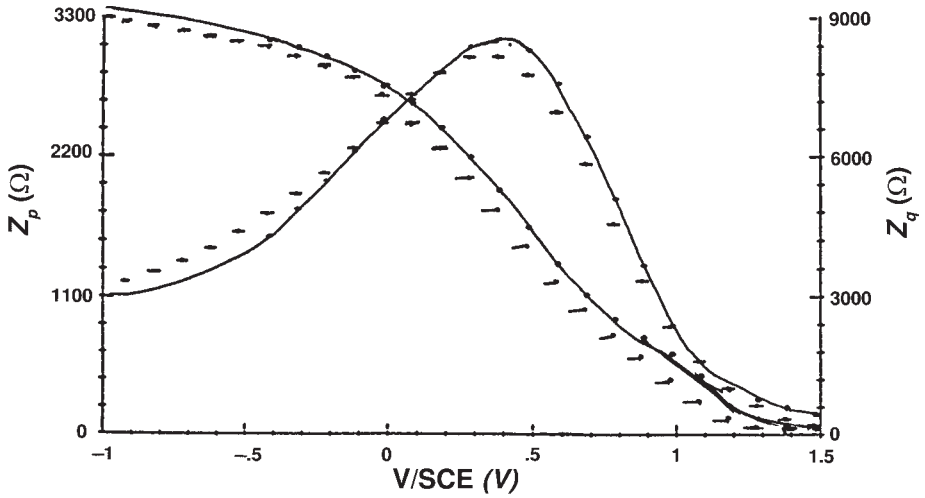

Fig. 2. Comparison between the electrochemical (solid lines) and optoelectrochemical (broken lines) impedance curves. $F = 1$ kHz, v = 9 mV, light power $P = 0.6$ μW/cm^2.

Optoelectrochemical Impedance on EDS Structure

Another way to perturb the EDS structure is to use a small modulated light (Fig. 2). The photon excitation of the semiconductor with energy larger than the band gap induces a photocurrent into the structure and generates a photovoltage. If the light intensity is weak enough to keep the structure close to its initial thermodynamic equilibrium position, the transfer function between the photocurrent and the photovoltage is an impedance term that we note as optoelectrochemical impedance *(4)*.

$$V_{ph} = Z_{opto} J_{ph}$$

Assuming a neglected attenuation of light flux in the electrolyte and through the dielectric, and a diffusion length >1 (as in the case of silicon), the photocurrent can be expressed as follows:

$$J_{ph}(t) = q\phi(t)(1 - R_\lambda)$$

in which $\phi(t)$ is the light flux reaching the semiconductor and is proportional to the light power $P(t)$; and R_λ is the reflection coefficient of the semiconductor.

Figure 2 shows the optoelectrochemical impedances recorded on p-type Si/Si_3N_4 (50 nm)/1 *M* NaCl solution for a 1-kHz frequency under a light power of 0.6 mW/cm^2. The electrochemical impedances have been obtained on this structure in similar experimental conditions but with electrical modulated excitation. Because optoelectrochemical impedance measurements were performed with an open circuit whereas electrochemical impedance concerns a closed circuit, the impedance contributions relative to the oxide layer and the electrolyte have been subtracted in the last case for direct comparison. Whatever the type of perturbation (electrical or

light), the resulting impedance curves exhibit a similar shape where the three polarization domains can be distinguished. This result is consistent while the light excitation is low. Note that just a small shift between the curves can be attributed to a low change in Fermi level position in the semiconductor owing to the light. This shift remains constant if the measure is made with a constant light intensity.

Under high light intensity, the semiconductor deviates from its quasi-equilibrium state, but only the in-phase impedance is strongly affected. This is important because the detection of charge effects on blocking structures requires knowing only the location of the Z_q curve with respect to the potential axis. By analogy with electrical impedances, this means that even under strong illumination, optoelectrochemical impedance allows the flat band potential change induced by surface charge modifications to be followed.

Materials and Methods

To detect eventual electrical behavior modifications of the structure induced by biological recognition mechanisms, electrochemical impedance measurements were performed on EDS structures in which various biological species were immobilized. Two types of biochemical systems based on affinity interactions were investigated: the antibody/antigen recognition and the hybridization of two complementary oligonucleotides.

Preparation of Samples

All the Si/SiO_2 substrates were cleaned by immersing in boiling methanol for 10 min, rinsing with deionized water, dipping in boiling acetone for 10 min, and air-drying at room temperature. The generation of OH groups on the silica surface was done by treating the substrates in concentrated sulfochromic acid for 3 min and rinsing with deionized water.

Before the immobilization of IgG antibodies (monoclonal anti-α-fetoprotein from Biomerieux), the SiO_2 surface was grafted with a heteropolysilsesquioxane layer synthesized from two trifunctional monomers, aminopropyltrimethoxysilane and butyltrimethoxysilane, according to the process described in ref. *5*. Then IgG antibodies were fixed on the amino surface through the disuccinimidyl suberate homobifunctional coupling reagent. Finally, the grafted surfaces were incubated at room temperature for 1 h in a 0.1 *M* phosphate-buffered saline (PBS) solution containing different specific antigen (α-fetoprotein from Behring) concentrations (0.01–1 μg/mL). The electrodes were carefully rinsed with pure PBS. The electrochemical impedances of the structures were measured in the experimental cell containing only 0.1 *M* PBS solution for a 100-kHz frequency before and after antigen incubation.

Oligonucleotides were immobilized after the hydroxylated Si/SiO_2 electrode surfaces were coated with 2.5 μL of a 5% aqueous solution of 3-aminopropyltriethoxysilane (APTS) (Sigma, St. Louis, MO) and air-dried

at room temperature for 1 h. Excess APTS was removed by rinsing the modified electrode with Tris-HCl buffer, pH 7.1, for 20 min. Single-stranded oligos were coupled to the APTS-modified electrode using the *N*-bromosuccinimide method reported by Keller et al. *(6)*. The strands used for immobilization were all synthetic oligomers (single-stranded DNA [ssDNA]) of either $(dT)_{20}$ or $(oligo\ 442)_{18}$ (CAG CAG CCG CGG TAA TAC). Twenty microliters of an aqueous solution of 0.01 *M N*-bromosuccinimide was added to 1 mL of a 1 mg/mL solution of ssDNA in 1 *M* aqueous $NaHCO_3$ and left to react for 15 min at 0°C. A 2.5-μL sample of *N*-bromosuccinimide-DNA solution was then deposited onto the APTS-derivatized electrode and left to dry overnight in a saturated atmosphere. The modified electrode was then rinsed with Tris-HCl buffer. The complementary strands used for hybridization were poly(dA) (1000 bases) and *Escherichia coli* chromosomic DNA (25 kilobases) containing several complementary sequence fragments of the (oligo 442). Noncomplementary poly (dC) (1000 bases) and plasmid DNA (99 kilobases) were also used.

Results and Discussion

Figures 3–5 present the results of electrical impedance measurements performed for various probe/target biological systems. Figure 3 gives the impedance curves obtained on the structure modified with antibodies before and after antigen incubation. It appears that the in-phase impedance curves were very dependent on the quantity of antigen, particularly in the depletion domain, whereas the out-of-phase impedance curves seem not to be affected by the antigen incubation. Note that the Z_q and Z_p curves are strictly in the same position in regard to the applied potential before and after the antibody/antigen interactions.

Figure 4 shows the impedance curves recorded on a structure modified with single DNA $(dT)_{20}$ strands before (curve 1) and after (curve 2) hybridization with complementary oligo poly (dA). Only the out-of-phase impedance curves were reported here because the main effect after the hybridization process is the shift in the curves along the potential axis. The move of the curves toward negative potentials was systematically observed after incubation of the electrode in solution containing complementary strands. Curve 3 shows that this shift was reversed after the denaturation process. Successive hybridization-denaturation steps made on the same electrode gave reproducible shifts of the Z_q curves until the electrode was altered by the different treatments. This means that the DNA immobilization process must be improved, particularly for the grafting of silane, but there was a sufficient physical binding of DNA strands on the surface to perform experiment. When electrodes with immobilized $(dT)_{20}$ were put in contact with solution containing noncomplementary poly (dC) at the same concentration, no shifts were observed (Fig. 5).

Such shifts have also been recorded when using $(dA)_{18}$ complementary strands *(7)*. When the electrodes with immobilized oligo (442) probes

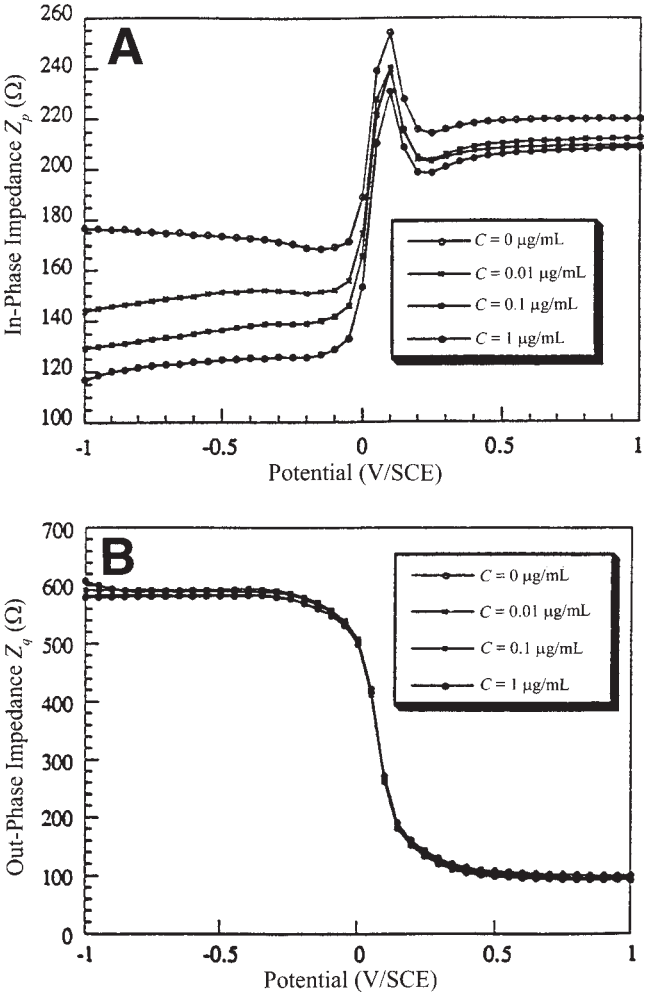

Fig. 3. **(A)** In-phase and **(B)** out-of-phase impedance curves performed on (pSi/SiO_2/grafted antibodies) in 0.1 *M* PBS solution for various concentrations of antigen in the incubation solution. *C* = 0 μg/mL means incubation in horse serum without antigen, and (*C* = 0.1 μg/mL means incubation in horse serum with antigen.

were exposed to solutions containing complementary targets (either *E. coli* or complementary DNA polymerase chain reaction [1000 bases]), similar results were obtained. In the same way, no significant shifts have been recorded when solution contains no complementary strands (plasmid) *(8)*.

These shifts observed systematically after the hybridization steps and the reverse move obtained after the denaturation process allow us to correlate them to the biological recognition process. The moves of the curves correspond to changes in the flat band potential values in the underlying semiconductor in response to surface charge modifications. This is consistent with the fact that oligonucleotides bear negative charges on phosphate groups (Fig. 6). When recognition between immobilized oligonucleotides

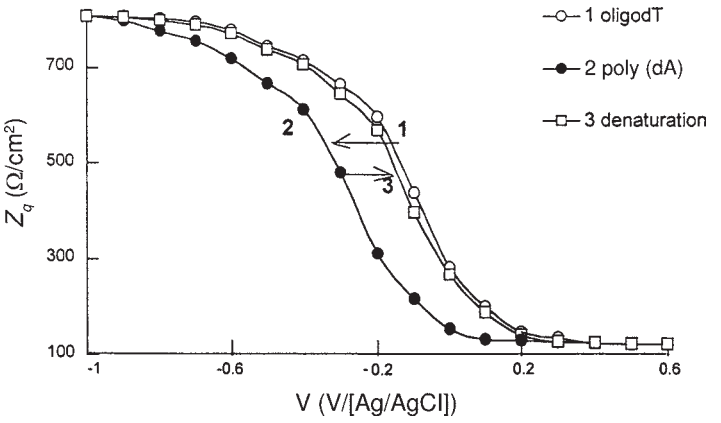

Fig. 4. Out-of-phase impedance curves performed on (pSi/SiO_2/immobilized oligo [dT]) in Tris buffer. 1, Before hybridization; 2, after hybridization with poly (dA); 3, after denaturation step.

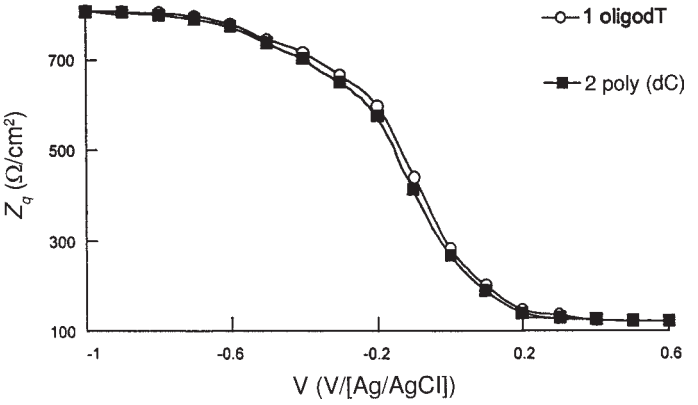

Fig. 5. Out-of-phase impedance curves performed on (pSi/SiO_2/immobilized oligo [dT]) in Tris buffer. 1, Before hybridization; 2, after incubation with poly (dC).

and their complementary strands occurs on the surface, there is formation of a duplex leading to an increase in negative charges at the oxide/electrolyte interface. Because the working electrode is a blocking structure and considering an ideal Si/SiO_2 interface and no interacting charges inside the oxide layer, the change in charges at the interface induces an equivalent variation of charge of opposite sign in the space charge layer of the underlying semiconductor. This new repartition of charges inside the semiconductor layer near the Si/SiO_2 interface provokes a change in the flat band potential value resulting in displacement of the impedance curves along the potential axis. According to this charge effect, when the hybridization process cannot take place (in the case of noncomplementary strands), no shifts are observed. This analysis is reinforced if we consider the results obtained with the α-fetoprotein/anti-α-fetoprotein system. These antigens

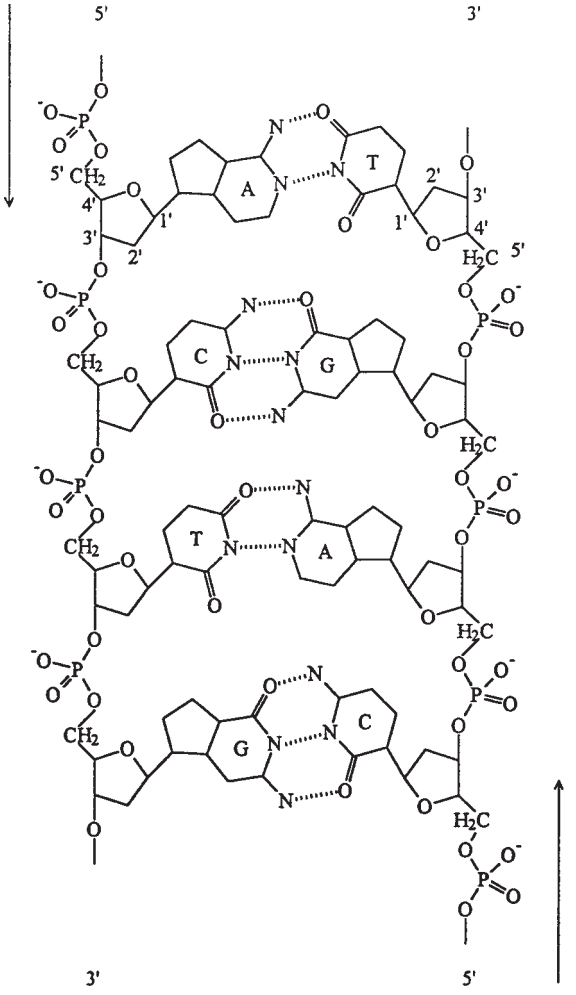

Fig. 6. Structure of DNA. The phosphate groups bear negative charges.

do not carry positive or negative charges on the surface during the recognition interactions and thus the impedance curves stay exactly at the same position.

As already described, the electrical behavior of such structures can also be analyzed thanks to the optoelectrochemical impedance measurements. The advantage of this method is that the modulated perturbation can be applied on well-defined areas of the surface. Only the lighted areas will be probed. Thus, measurements can be performed on the same wafer where 12 active areas were designed (Fig. 7). All these areas were cleaned and treated with sulfochromic solution (treatment a). Three of them (1–3) were left without other modifications, whereas the others were covered with APTS (treatment b). On the areas 7–12, oligos (442) were immobilized (treatment c) and only the areas *(10–12)* were put in contact with comple-

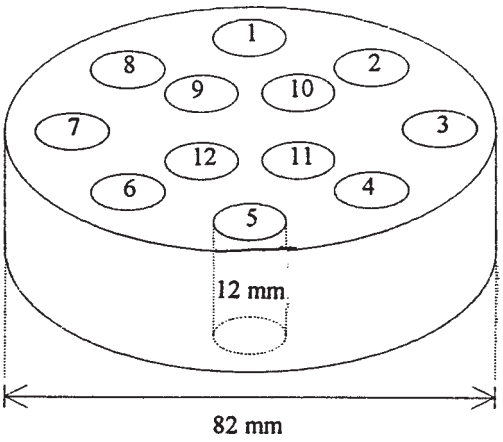

Fig. 7. Scheme of different active areas on the surface of the structure for optoelectrochemical impedance mapping. 1–3, Only cleaned and sulfochromic treatment (a); 4–6, treatment (a) + APTS grafting (b); 7–9, treatment (b) + immobilization of DNA single strands (oligo 442) (c); 10–12, treatment (c) + hybridization with complementary DNA strands (*E. coli*).

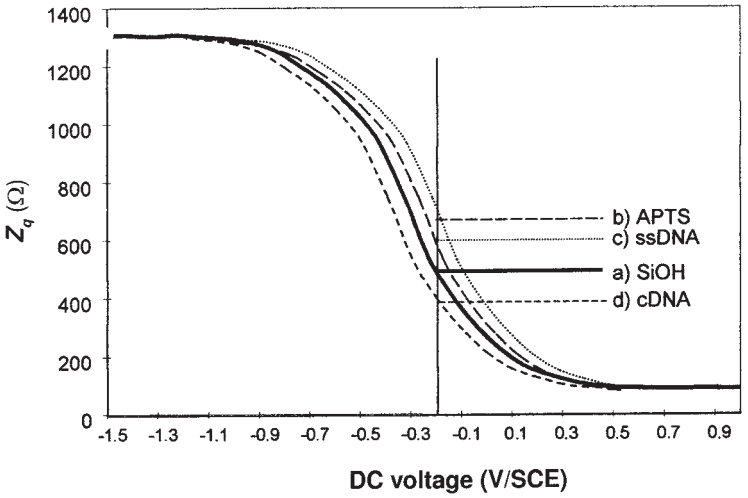

Fig. 8. Position of out-of-phase curves as function of treatment. Curve a is an experimental curve recorded on surface after treatment a. This curve allows the determination of the best potential to apply for obtaining the most sensitivity. The others curves are drawn from Fig. 8. This representation gives evidence of the shifts of flat band potential.

mentary DNA strands for the hybridization process (treatment d). Experimental measurements consisted of recording the optoelectrochemical impedance curves as a function of the applied potential on the areas (1–3) considered as reference (curve a, Fig. 8). Then, an optoelectrochemical impedance mapping was performed by scanning the modulated light probe

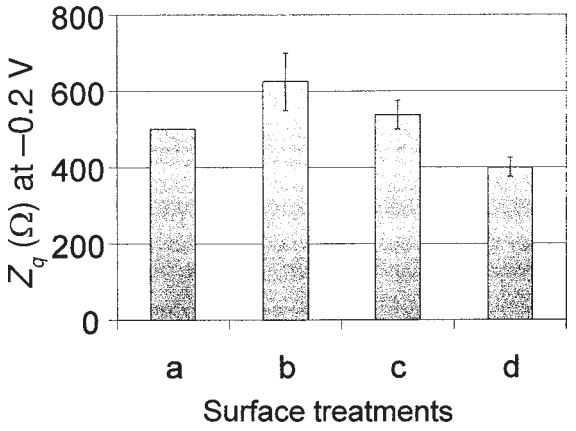

Fig. 9. Variation in the out-of-phase optoelectrochemical impedances as function of treatment. Measurements were performed at V = –0.2 V. Each column represents the average Z_q value calculated from the three circles with the same treatment (20 measured points/per active area).

on the overall surface of the wafer for a fixed applied potential chosen in the depletion domain (V = –0.2 V/[Ag/AgCl]) (Fig. 8). This value corresponds to a strong slope in the decreasing part of the Z_q curve and allows great sensitivity to the Z_q variations. With similar treatments, each of the three areas gave about the same values of Z_q. By contrast, the experimental Z_q values were dependent on the surface modifications. As shown in Fig. 9, the Z_q values decreased after the immobilization of DNA single strands and decreased further after the hybridization. If these decreases in Z_q are reported on the (Z_q, V) diagram, we can draw the corresponding impedance curves with respect to the potential axis (curves b–d, Fig. 8) and connect these Z_q variations to the changes in flat band potential. In agreement with the previous results, the Z_q curves were shifted toward negative potentials after the hybridization step.

These results are quite important because they show the opportunity to perform many measurements on the same structure variously modified on its surface. In particular, if we consider the DNA chips, this method seems very promising and appears to be an alternative way to detect directly the DNA hybridization on a localized point, without the use of labeled probe.

Conclusion

Both the electrochemical and optoelectrochemical impedance measurements were shown to be a powerful tool for the detection of the hybridization process between two complementary DNA strands on the surface. The shift of the impedance curves with respect to the potential axis is interpreted in terms of the variations in the charges at the interface induced by the DNA recognition. Regarding multidetection on the same wafer, the

electrochemical impedances require as many independent electrical contacts as active areas. The use of the light perturbation (allowing the optoelectrochemical impedance measurement) presents a serious advantage because of its ability to probe the surface easily by scanning and to perform local measurement on a very simplified structure. Only an ohmic contact is required on the rear face. In the future, this technique of direct detection of the hybridization can be adapted to DNA chips.

Acknowledgments

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