Carbon nanotubes field effect transistors biosensors

Biosensores con transistores de efecto de campo basados en nanotubos de carbono

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Abstract

Carbon nanotube transistor arrays (CNTFETs) were used as biosensors to detect DNA hybridization and to recognize two anabolic steroids, stanozolol (Stz) and methylboldenone (MB). Single strand DNA and antibodies specific for STz and MB were immobilized on the carbon nanotubes (CNTs) in situ in the device using two different approaches: direct noncovalent bonding of antibodies to the devices and covalently trough a polymer previously attached to the CNTFETs. A new approach to ensure specific adsorption of the biomolecules to the nanotubes was developed. The polymer poly (methylmethacrylate$_{0.8}$-co-poly (ethyleneglycol) methacrylate$_{0.8}$-co-N-succinimidyl methacrylate$_{0.1}$) was synthesized and bonded noncovalently to the nanotube. Aminated single-strand DNA or antibodies specific for Stz and MB were then attached covalently to the polymer. Statistically significant changes were observed in key transistor parameters for both DNA hybridization and steroids recognition. Regarding the detection mechanism, in addition to charge transfer, Schottky barrier, SB, modification, and scattering potential reported by other authors, an electron/hole trapping mechanism leading to hysteresis modification has been determined. The presence of polymer seems to hinder the modulation of the electrode-CNT contact.

Resumen

Matrices de transistores de efecto de campo basados en nanotubos de carbono (CNTFETs) fueron usados como biosensores para la detección de la hibridación de ADN y para el reconocimiento de dos esteroides anabolizantes, estazonolol (Stz) y metil-boldenona (MB). Secuencias simples de ADN y anticuerpos específicos para Stz y MB fueron inmovilizados sobre los nanotubos de carbono, (CNTs), in situ en los dispositivos usando dos estrategias diferentes; enlace directo no-covalente y enlace covalente a un polímero previamente fijado a los CNTFETs. Se ha desarrollado una nueva estrategia para asegurar la adsorción especifica de las biomoléculas a los CNTs. Se sintetizó el polímero poli (metilmetacrilato$_{0.8}$ -co-(polietilenglicol) metacrilato$_{0.8}$-co-N-succinimidil metacrilato$_{0.1}$) que fue enlazado no-covalentemente a los CNTs en el dispositivo. Posteriormente una secuencia simple de ADN o en su caso el correspondiente anticuerpo específico para Stz o MB fueron enlazados covalentemente al polímero. Se observaron cambios estadísticamente signiticativos en parámetros clave del transistor tanto para la hibridación de ADN como para el reconocimiento de los esteroides anabolizantes. Respecto al mecanismo de detección, además de transferencia de carga, modificación de la barrera de Schottky, SB, y dispersión de potencial publicados en la bibliografía, se ha detectado un mecanismo de atrapamiento de electrones y huecos que lleva a una modificación de la histéresis. La presencia del polímero parece impedir la modulación ejercida por los contactos electrodo-CNT.

1. Introduction

The broad applications of nanotechnology make it one of the most rapidly growing areas of research in contemporary science and medicine [1,2]. The unique chemical and optical properties of nanomaterials in terms of particle aggregation, photoemission, electrical and heat conductivity, and catalytic activity have paved the way for development of nanobioelectronic devices [3,4]. Micro- and nanofabrication has allowed the production of ultrasensitive, portable, and inexpensive biosensors. These devices generally rely on chemical or biological receptors that recognize a particular compound of interest and transfer this recognition event effectively [5].

The development and utilization of micro- and nanostructured materials, new semiconductor [6], quantum dots [7], plasmonic nanoparticles [8], nanowires [9] magnetic nanoparticles [10] and CNTs [11] have promoted extensive research activity in the field of biosensing, taking advantage of the many novel phenomena that occur at nanoscale.

Recently, one-dimensional nanostructures, such as carbon nanotubes and semiconductor nanowires, have been successfully demonstrated to be sensitive biological sensors [12]. CNTs show unique features that are being used for the development of nanometer scale materials with outstanding potential technological applications [13]. Owing to their structural and electronic uniqueness, carbon nanotubes have been proposed either in advanced electrochemical devices or as molecular-sized electrodes for very fast electrode kinetics research and for sensing and immunosensing.

One promising approach is the direct electrical detection of biological macromolecules using semiconducting nanowires or CNTs configured as field-effect transistors, CNTFETs, which change conductance when charged macromolecules are bound to receptors linked to the device surfaces [14-17]. CNTFET immunosensors have been prepared by joining the antibodies directly to the CNT or through the use of aptamers. Park et al. [18], have immobilized monoclonal antibodies against carcinoembryoic antigen. Chen et al.[19] have developed CNTFETs for biomarker detection for the early diagnosis of cancer. Li et al.[20] have demonstrated a novel FET immunosensor for the

detection of prostate-specific antigen. Utilizing a monoclonal antibody against immunoglobulin (IgE) immobilized in the device, the IgE concentration was estimated using a CNTFET [21]. Likewise, the possibility of a label-free electrical detection of the DNA-hybridization utilizing semiconductor field-effect sensors offers a new approach for a new generation of DNA chips with direct electrical readout for a fast, simple and inexpensive analysis of nucleic acid samples. Poghosian [22] has evaluated the possibilities and limitations of label-free detection of DNA hybridization with field-effect-based devices. The inherent miniaturization of such devices and their compatibility with advanced microfabrication technology make them very attractive for DNA diagnostics.

This paper report a summary of the previously reported [23, 24] label free detection of DNA hybridization and recognition of two anabolic androgenic steroids, using a large array of CNTFET. We present a methodology for avoiding nonspecific DNA adsorption on CNTs using a polymer, Figure 1, anchored to the device and providing at the same time a stable binding for DNA and specific antibodies for Stz and MB through robust amide linkages.

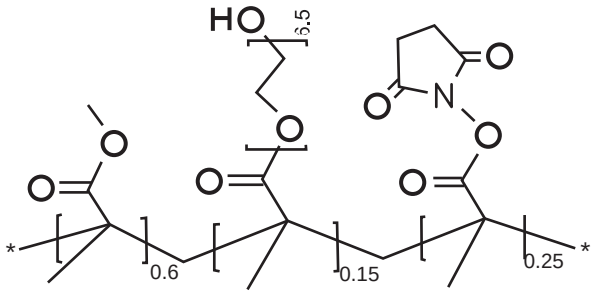
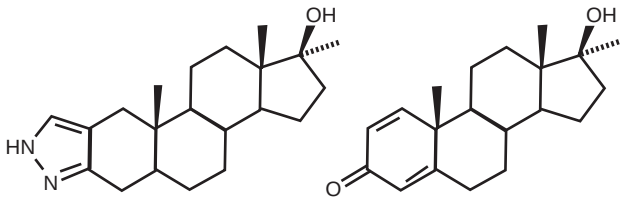


Figure 1. Poly (methylmethacrylate$_{0.6}$-co-poly (ethyleneglycol) methacrylate$_{0.15}$-co-N-succinimidyl methacrylate$_{0.25}$).
Figura 1. Poli (metilmetacrilato$_{0.6}$-co-poli (etileneglicol) metacrilato$_{0.15}$-co-N-succinimidil metacrilato$_{0.25}$).

Anabolic androgenic steroids (AAS) are banned substances in different fields. The World Anti-Doping Agency (WADA) [25] and the European Commission [26] have prohibited the utilization of AAS compounds for enhancing athletic performance and as a growth promoter in cattle owing to their being considered a public health risk. In this paper, we propose the utilization of field-effect transistor arrays based on carbon nanotubes as immunosensors for the detection of stanozolol (Stz) and methylboldenone (MB), Figure 2. Stz and MB are two AAS that are banned by WADA and the European Commission. The methodology used takes advantage of the specifity of the immune assays but with the added value of the electronic measurements being faster and direct. We report and analyze the changes of the electrical CNTFET characteristics upon interaction with the chemicals used for binding the DNA and upon hybridization.



Stanozolol (St) Methylboldenone (MB)

Figure 2. Anabolic steroids: Stanozolol and Methylboldenone (MB).
Figura 2. Esteroides anabolizantes: Estanozolol y Metil-Boldenona.

2. Experimental

The experimental details for the fabrication of the sensors arrays, the synthesis of the polymer and the production of the specific steroids were reported previously [23, 24]. Oligonucleotide NH2-ssDNA (5'-NH2-hexyl-CGAGTCATTGAGTCATCGAG-3') and its complementary C-ssDNA (5'-CTCGATGACTCAATGACTCG-3') were used. Two strategies were followed for the bonding of the biomelecules in the CNTFET. For the first one, the direct bonding of the DNA and specific antibodies to the CNTs in the CNTFET as represented in Figure 3 was carried out. For the second one, the polymer was bonded to the CNTs no-covalently and afterwards, aminated sequences of DNA and the antibodies were bonded to the transistors by covalent strong amide linkages, Figure 4.

The devices were electrically characterized before and after attaching any chemicals or biomolecules in order to determine their effect on the electrical characteristics of the transistors, and statistical analyses of a large array of devices were performed. Only devices having good transistor behaviour showing on/off current ratio higher than 100 were considered in the study. All the bare devices show p-type or ambipolar transistor behavior.

The parameters of interest are the threshold voltage VTH and the drain-source current IDS at the maximum negative voltage. The threshold voltage VTH is taken as the x-intercept of the line tangent to the steepest part of IDS-VG curve. Because the threshold voltage depends on the sweep direction of the back gate voltage, a separate VTH is extracted for the forward and the reverse sweep.

3. Results and discussion

Figure 5 shows the changes in the I_{DS}-V_G curves in the DNA hybridization process on a typical CNTFET. The plots of the device show how the deposition of the chemicals and biomolecules modify the I_{DS}-V_G

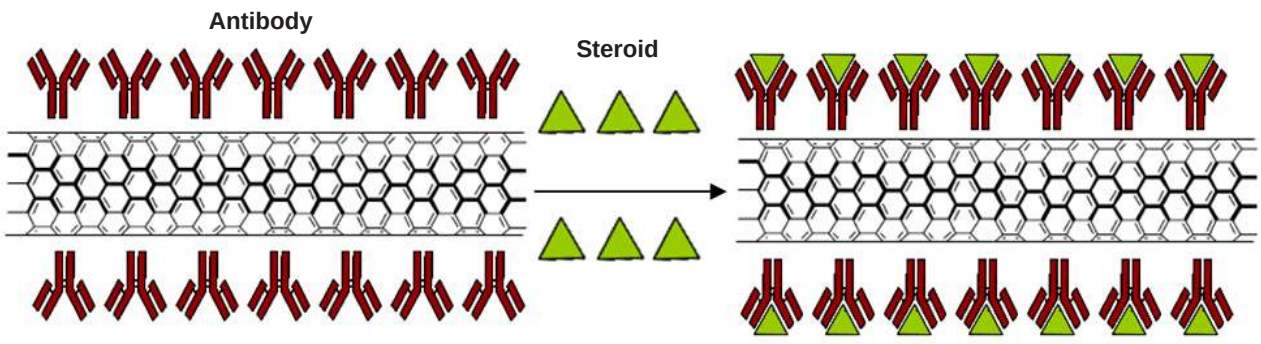


Figure 3. Schematic representation of the bonding of antibodies and steroids to CNTs.
Figura 3. Representación esquemática del enlace de anticuerpos y esteroides a los CNTs.

and/or the V_{TH} characteristics. The polymer was used to prevent non-specific adsorption of the other biomolecules, and the last two steps shown in Figure 5 involve the blocking of excess succinidimyl groups using ethanolamine (EA) blockers, followed by hybridization. It can be seen from Figure 5 an important shift of the VTH with the hybridization as well as significant decrease in I_{DS}.

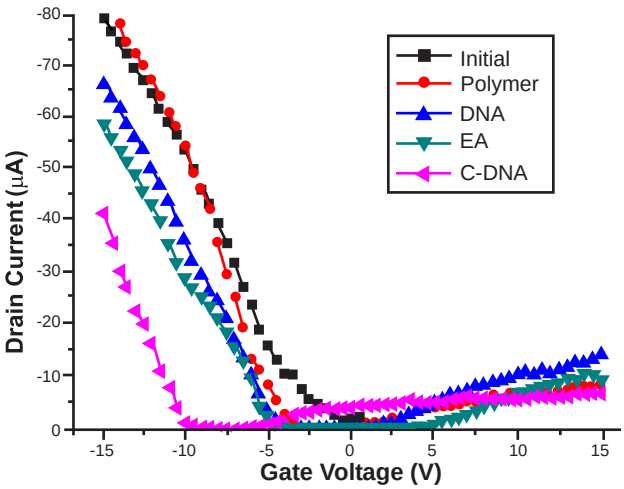


Figure 5. I_{DS}/V_G plots of a typical device for the direct swept of: the bare device, after anchoring the polymer, after simple sequence DNA bonding, after blocking the succynimidil groups with EA and in the hybridization step with the complementary sequence.
Figura 5. Curvas I_{DS}/V_G de un dispositivo típico para el barrido directo de: el dispositivo de partida, después del anclaje del polímero, después del enlace de la secuencia simple de ADN, bloqueo de los grupos succinimidil con EA y en la hibridación de ADN.

Likewise, when the forward and reverse sweep are considered, it was determined [23] a large increase in the hysteresis in the I_{DS}/V_G curves following DNA hybridization. The shift of V_{TH} to more negative values for the forward sweep (V_G from -15V to +15V) indicates electron traps, whereas the shift to more positive values for the reverse sweep (V_G from +15V to -15V) is evidence of holes trap. It is known that both electron and hole transport can occur on a double strand DNA, with holes hopping on guanine and adenine bases, and electrons on cytosine and thymine bases [27].

Regarding the steroids recognition, Figure 6 shows the I_{DS}/V_G plots for the recognition step when the antibodies are bonded to the polymer. When polymer is used to bond the antibodies, the changes in IDS are statistically significant for the forward and reverse steps in the absence of polymer in Stz recognition and in the presence of polymer for the MB recognition. The presence of polymer reduces considerably the hysteresis and the contribution of SB as indicated by the reduction of the changes in the mean IDS values in Stz recognition. In the MB recognition in the absence of polymer, the changes in IDS are very low probably because the changes in SB are balanced by those of the scattering potential. The presence of polymer seems to prevent the changes in SB and makes visible the changes in IDS probably due to potential scattering for the MB recognition.

With regard to the mechanism of detection in the absence of polymer, several overlapped mechanisms could be present. In addition to charge transfer, scattering potential and SB modification reported by other authors [28] electron and hole trap mechanisms with hysteresis decrease in the absence of polymer, and hysteresis increase in the presence of polymer have been determined as for the electronic detection of DNA hybridization.

In the presence of polymer, the changes in SB are possibly of low relevance, making the scattering mechanism and charge transfer the predominant detection mechanisms.

The bonding of the polymer with the CNTFETs prevents the nonspecific adsorption of the steroids such as nantranone and antibodies on the CNTFETs, but it has been determined that streptavidin causes statistically significant changes, indicating nonspecific adsorption to the polymer or some kind of conformational changes that produce electron transfer in the transistor. Nonspecific binding is a big challenge particularly in the immunoassay field.

4. Conclusions

The electronic detection of the DNA hybridization has been carried out by using a large array of CNTFETs. The method uses a synthetic polymer that is well adsorbed to the walls of CNT and carries activated succinimidyl ester groups used to fix the NH2-ssDNA probes. This method of anchoring the probe DNA can prevent the nonspecific adsorption of DNA molecules onto the CNTFET that can occur by virtue of aggregation on the sidewalls of the contact electrodes. The mechanism of the CNTFETs' electrical response is changed significantly by the utilization of the polymer. DNA hybridization produced statistically significant changes in the threshold voltages reflecting the charge trapping character of hybridized DNA. Through these observations it has been possible to detect the charge transfer inherent to the hybridization reaction.

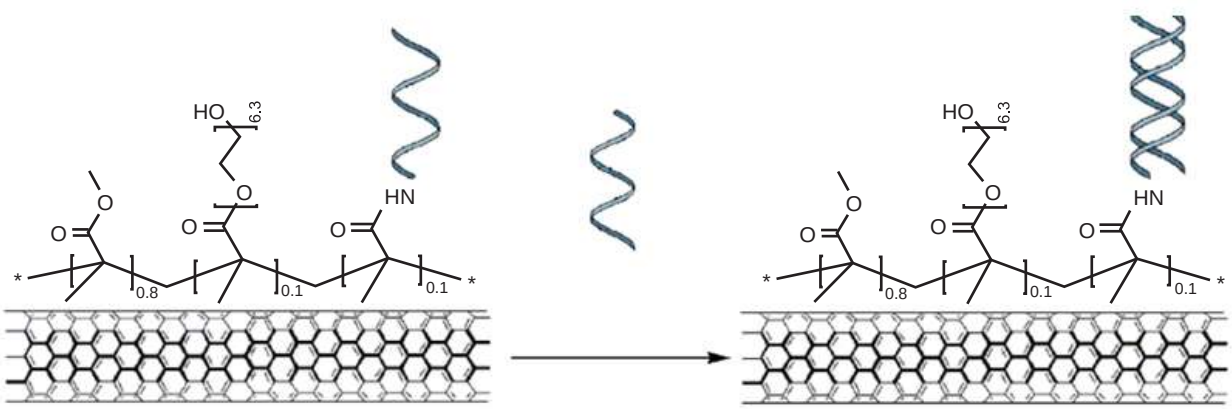


Figure 4. Schematic representation of the DNA hydridization after bonding the aminated DNA single sequence to the polymer in the CNTFET.
Figura 4. Representación esquemática de la hibridación de ADN después del enlace de la secuencia simple de ADN aminada al polímero.

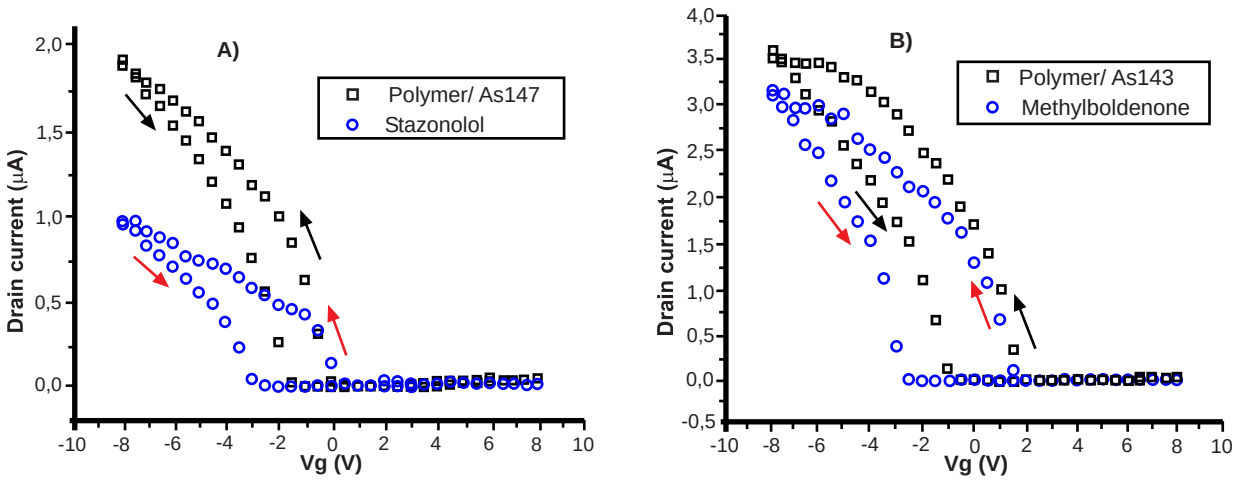


Figure 6. I_{DS}/V_G plots of typical CNTFETs. (A) After anchoring the polymer and As147 antibody (black) specific for Stz and after anchoring stanozolol (blue). (B) After anchoring the polymer and As143 antibody (black) specific for MB and after anchoring methylboldenone (blue).
Figura 6. Curvas I_{DS}/V_G de un CNTFET representativo. (A) Después del anclaje del polímero y el anticuerpo , As147, especifico para el Stz (negro) y después del anclaje de Stz (azul). B) Después del anclaje del polímero y el anticuerpo As 143, específico para la MB (negro) y tras el enlace con la MB (azul).

The feasibility of electronic detection of two anabolic steroids, stanozolol and methylboldenone, has been demonstrated using CNTFET transistors. The bonding of Stz and MB to the specific antibody produces strong changes in the electrical properties of the CNTFET, showing statistically significant changes in the Vth in the forward and reverse sweeps in the recognition step when the antibody is attached directly to the CNT and changes in the forward sweep of the recognition step when the antibody is bonded with the polymer, indicating charge transfer mechanism.

As far as the specificity of the detection is concerned, it has been determined that the CNTFETs do not show statistically significant changes when the antibodies are in contact with other steroids such as nantranone; nevertheless, cross-reactivity with other steroids will have to be studied more exhaustively. The data reported represent a proof of concept of the feasibility of electronic steroid immune detection. Nevertheless, further research needs to address the development of practical biosensors that prevent nonspecific adsorption of biomolecules such as streptavidin on CNTs and enable direct detection, thus avoiding the necessary separation steps when serum samples are used.

5. Acknowledgments

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