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Geometry-dependent DNA-TiO_2 immobilization mechanism: A spectroscopic approach

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ABSTRACT

DNA nucleotides are used as a molecular recognition system on electrodes modified to be applied in the detection of various diseases, but immobilization mechanisms, as well as, charge transfers are not satisfactorily described in the literature. An electrochemical and spectroscopic study was carried out to characterize the molecular groups involved in the direct immobilization of DNA structures on the surface of nanostructured TiO_2 with the aim of evaluating the influence of the geometrical aspects. X-ray photoelectron spectroscopy at O1s and P2p core levels indicate that immobilization of DNA samples occurs through covalent (P—O—Ti) bonds. X-ray absorption spectra at the Ti2p edge reinforce this conclusion. A new species at 138.5 eV was reported from P2p XPS spectra analysis which plays an important role in DNA-TiO_2 immobilization. The P—O—Ti/O—Ti ratio showed that quantitatively the DNA immobilization mechanism is dependent on their geometry, becoming more efficient for plasmid ds-DNA structures than for PCR ds-DNA structures. The analysis of photoabsorption spectra at C1s edge revealed that the molecular groups that participate in the C1s → LUMO electronic transitions have different pathways in the charge transfer processes at the DNA-TiO_2 interface. Our results may contribute to additional studies of immobilization mechanisms understanding the influence of the geometry of different DNA molecules on nanostructured semiconductor and possible impact to the charge transfer processes with application in biosensors or aptamers.

1. Introduction

DNA nucleotides are used as molecular recognition system, double stranded deoxyribonucleic acid (ds-DNA) is a well characterized system, a double helix formed when two single strands are paired by hydrogen bonds to form a long chain of nitrogenous base pairs, Adenine-Thymine and Cytosine-Guanine (A-T and C-G). A single strand DNA (ss-DNA) is formed by a polyanionic sugar-phosphate backbone connected to the nitrogenous bases, rich in π-bonds. This is a system that contains a stack of π-orbitals extending within the DNA, facilitating electron transfer, which is one of the reasons for the various applications of DNA structure in biosensors devices [1,2].

DNA-based biosensors consist of the immobilized DNA strand to detect the complementary nucleic acid sequence by DNA target hybridization. DNA immobilization on the surface of electrodes can be applied in the detection of viral diseases [3–5]. These detection devices depend on the efficiency of charge-transfer processes (electric signal) occurring from the complementary and/or adjacent nucleobases of the tris-phosphate groups to the conductor or semiconductor substrate. Understanding the characteristics of the immobilization of different geometrical structures of DNA it is possible to deepen into the charge transfer mechanisms relevant for application in DNA-based biosensors. The interaction of biomolecule and the substrate surface involve covalent, ionic, hydrogen or van der Waals bonding that depends on the molecular structure, size and the nanostructured metal oxide properties (i.e. surface charges, energy, functional groups, etc.) [6,7].

TiO_2 is widely used as a semiconductor material functionalized with molecules due to its high biocompatibility, high stability, easy availability and low cost [8,9]. Connor et al. have demonstrated that the phosphate groups perform electrostatic interactions with TiO_2 using internal reflectance spectroscopy (IRS) [10], and Liu et al. studied the photo-oxidation of ds-DNA immobilized on TiO_2 films by cyclic voltammetry [11].

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This work aims to study chemical species involved in the mechanisms of direct immobilization of the *Escherichia coli* plasmid ds-DNA (pDNA) and PCR ds-DNA (PCR) on TiO_2. For this purpose, a combination of electrochemical and core-level spectroscopic characterizations was performed. Our results showed that these DNA molecules are immobilized on TiO_2 surface through P—O—Ti bonds, and that immobilization efficiency depends on the DNA geometry. The charge transfer process between DNA molecules and TiO_2 substrate were elucidated by cyclic voltammetry and corroborated by C K-edge and Ti L-edge X-ray absorption.

2. Experimental

2.1. Materials and Methods

Titanium (IV) isopropoxide (97%), ethanol (99.5%) and hydrochloric acid (37%) were purchased from Sigma-Aldrich. ITO (Indium Tin Oxide) coated on glass (75 × 50 × 0.7 mm, surface resistivity 15 Ω/sq) was obtained from (Lumtech, Taiwan). Tris hydrochloride (Tris-HCl) solution, Ethylenedinitrilotetraacetic acid (EDTA) buffer, Tris-EDTA buffer solution, glucose solution, sodium hydroxide P.A., sodium dodecyl sulfate solution, sodium hypochlorite solution, potassium acetate, glacial acetic acid, chloroform, and isopropanol were purchased from Sigma-Aldrich. dNTP Set was acquired from Thermo Scientific and Platinum Taq DNA Polymerase were acquired from Invitrogen™.

2.2. dsDNA Samples (gDNA, pDNA and Amplicon)

The pCR™ 2.1-TOPO® (Invitrogen by Thermo) plasmid vector (pDNA) extraction was performed through the alkaline lysis method [12]. The 200 bp amplicon was obtained copying a known fragment of pDNA though the Polymerase Chain Reaction (PCR) method using the Taq DNA Polymerase Platinum kit (Invitrogen™ by Thermo Scientific) and M13 primers, following the manufacturer's recommendations. The sequence of nitrogenous bases from a plasmid strand and PCR-DNA are found in Supplementary material of this paper.

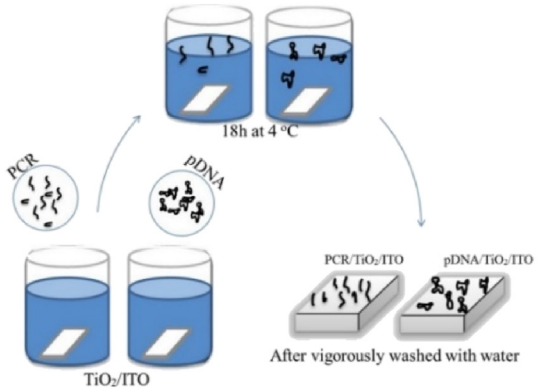
2.3. TiO_2 Films and Synthesis

The nanostructured TiO_2 was synthesized through the sol-gel method according to the literature [13]. A container containing 2.3 mL of Titanium (IV) isopropoxide, 7.6 mL of ethanol and 15.0 mL of hydrochloric acid was stirred for 6 h. The TiO_2 films were produced by adding an aliquot of the sol-gel of TiO_2 over ITO (Indium Tin Oxide)/glass substrates (15 × 15 mm) using the spin coating method at 3000 rpm by 60 s. Subsequently, the TiO_2/ITO films were sintered in a muffle at 350 °C for 45 min.

The surface morphology of the TiO_2/ITO film was obtained with an AFM (Innova, Bruker) on an area of (20 × 20) μm^2, operated in contact mode using silicon nitride cantilevers to scan the samples. They were performed with 512 × 512 pixels at a scan rate of 1.0 Hz. All measurements were performed at room temperature (296 ± 1 K) and 40 ± 1% relative humidity. The feedback control was adapted to the surface in order to obtain the best possible images, and they were analysed using the WSxM software [14] for determining the films surface roughness and thickness, as shown in in Fig. SI1 in Supplementary material. The films AFM images show a surface roughness of 1.02 nm and thickness of 15 nm, representative of a homogeneous surface.

2.4. DNA Immobilization

According to Scheme 1, the TiO_2/ITO films were immersed in two container containing 2.0 mL of deionized water, one with pDNA pellet, and another, with PCR pellet overnight at 4 °C for DNA immobilization. The DNA solutions were kept in phosphate buffer of pH 4. Afterwards, the films were vigorously washed with deionized water to remove the excess of DNA or DNA samples that have done weak bonds on the TiO_2 film surface, and dried at room temperature. The films were taken to an oven for 30 min at a temperature of 70 °C. The investigated samples will be labelled here as TiO_2/ITO, pDNA/TiO_2/ITO and PCR/TiO_2/ITO for nanostructured TiO_2 and immobilized DNA-TiO_2 films, respectively.



Scheme 1. DNA immobilization procedure on TiO_2/ITO surfaces in aqueous solution.

2.5. Electrochemical Experiments

The DNA immobilization was characterized by cyclic voltammetry (CV) in a conventional three-electrode configuration using the AUTOLAB PGSTAT 302N potentiostat (Metrohn Autolab, Utrecht, The Netherlands) equipped with GPES 4.9 software. The reference electrode was Ag/AgCl (3 M KCl) and the auxiliary electrode was a platinum wound wire. The working electrodes used in the experiment contained a total area of 15 × 10 mm ITO/glass, and the samples of interest were deposited at 10 × 10 mm. In the CV assays, a solution of methylene blue of 2.0 $\mu mol \cdot L^{-1}$ was used as electrolyte and intercalator at a scanning rate of 50 mV/s.

2.6. NEXAFS Experiments

Near edge X-ray absorption fine structure (NEXAFS) experiments were performed at the SGM beamline (250–1000 eV) at the Brazilian Synchrotron Light Source (LNLS), in Campinas, Brazil. Samples of TiO_2/ITO, pDNA/TiO_2/ITO and PCR/TiO_2/ITO were fixed on the sample holder with a carbon conductive tape and introduced into the ultra-high vacuum chamber (10^{-8} mbar). NEXAFS spectra were obtained in the total electron yield (TEY) mode at carbon, K absorption edge and titanium L-edge, before and after pDNA and PCR-DNA immobilization on nanostructured TiO_2. The spectra were normalized by the incident photon flux (I_0) measured with an Au mesh placed just upstream of the sample. Phenylalanine was used as standard reference for photon energy calibration of the NEXAFS spectra [15]. NEXAFS spectra shown in this work were background corrected by a linear pre-edge subtraction and linear regression beyond the edge.

2.7. XPS Experiments

X-ray photoelectron spectroscopy (XPS) measurements were performed at the ESCALAB 250Xi spectrometer (Thermo Scientific) equipped with an electron energy hemispherical analyzer using monochromatized Al Kα line ($h\upsilon = 1486.6$ eV) excitation. The spectra were energy referenced to the C1s signal of aliphatic C atoms at binding energy of 285.00 eV. XPS spectra were collected using X-ray beam spot size = 650 μm and with emission angle of 0° with respect to the sample surface. Survey spectra were measured from 0 to 1300 eV with pass energy of 50 eV and high resolution spectra acquired with 25 eV pass energy at C1s, N1s, O1s, Ti2p and P2p core levels. Sensitivity factors were