Nanomaterial-Based Biosensors for a Real-Time Detection of Biological Damage by UV Light*

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***Abstract*— In this work, the design and fabrication of a miniaturized and light-weight biosensor that can be used to monitor the biological effects of hostile ultraviolet radiation in earth and space are presented. The biosensor is generated by embedding a sensitive element to UV radiation, DNA, in a hybrid carbon-based nanomaterial. In particular, we present results on the fabrication and characterization of hybrid nanostructured films containing graphene nanoplatelets (GNPs) and double-stranded DNA for the *in situ* and real-time detection of UV radiation damaging effects from the changes of the film electrical properties induced by exposure to UV-C radiation. The biosensor is realized by the deposition of the sensitive unit GNP/DNA on a supporting substrate made of flexible polymers or glass.**

I. Introduction

Ultraviolet (UV) radiation from the sun, especially that characterized by shorter wavelengths and high frequencies, such as the UV-C band (wavelenght below 280 nm), has been epidemiologically and mechanistically linked to skin cancer and immunosuppression [1]. The most dangerous effect of UV-C exposure is the damage caused to DNA. In fact, the absorption spectrum of DNA has a peak around the wavelenght of 260 nm, which is determined by its nitrogen bases [2]. Recent studies on the behavior of DNA under UV-C irradiation (wavelenght 254 nm) showed that the UV destructive effect becomes evident after just one hour of irradiation [3]. This effect is determined by the formation of lesions on the nucleic acid sequence, which leads to the creation of covalent bonds between the pyrimidine bases and to the formation of cyclobutane pyrimidine dimers that determine the distortion of the DNA chain [4, 5].

The ozone layer in Earth's stratosphere has a fundamental role in allowing the development of different forms of life on earth, blocking the most energetic, and therefore dangerous, UV-C radiation [6]. However, man is increasingly exposed to the dangerous ultraviolet radiation, both on Earth, as a consequence of the ozone depletion in the atmosphere [7], and in space environment due to the predicted growing number of human exploration missions. For these reasons, the international scientific community is devoting much attention to study the consequences of the increased exposure to ultraviolet radiation by pursuing the development of biosensors [8-11] that could provide efficient solutions to monitor the effects of UV on biological systems. However, many of the available biosensors suffer from complex detection technology or lack of sensitivity. In this work, we describe the realization of a nanomaterial-based biosensor for UV-C radiation, which overcomes the limitations of traditional sensors by integrating a hybrid nano-assembly consisting of a biological molecule sensitive to UV-C radiation, the DNA, and graphene as signal transducer.

Graphene nanoplatelets (GNPs) are commonly used as fillers in composite materials to confer and enhance their electrical conductivity. In particular, GNPs have been replacing the more traditional carbon nanotubes (CNTs), due to their enhanced electrical and thermal properties, and to the greater exposed surface area that can show more advantageous for functionalization purposes [12]. In our work, the choice of DNA for the assembly of the GNP-based sensor surface has a dual function. On one hand, DNA is a biological target that responds in real time to UV exposure with structural changes. On the other hand, it acts as a dispersing agent for the highly hydrophobic graphene nanoplatelets.

So far, several studies have demonstrated that DNA can be an effective functionalizing agent for both carbon nanotubes [13], thanks to its ability of wrapping around the different geometries of nanotubes [14] , and for graphene as well. In this case, the DNA bases can intercalate within the GNP layers in a non-covalent interaction (van der Waals forces, and π-π bonds) that prevents the graphene layers from aggregating. In addition, the use of DNA as a functionalizing agent has the advantage of ensuring a good stability of the GNP dispersion without modifying the electrical and chemical properties of graphene [15, 16].

II. Materials and Methods

A. Materials

Graphene nanoplatelets with average thickness of about 60 nm and lateral size in the range of 3-7 μm (grade AO-4) were from Graphene Supermarket (USA). Double-stranded DNA (code 74782) was purchased from Sigma-Aldrich and used as received. DNA solutions and hybrid GNP/DNA dispersions were prepared in sterile deionized water (resistivity 18.2 MΩ·cm) from a Millipore Direct-Q3 UV water purification system. Biosensor supports were made of poly(dimethylsiloxane) elastomer (PDMS Sylgard 184, Dow Corning, USA) or glass microscope slides (extra-white glass, Menzel Gläser, Germany).

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Figure 1. Experimental setup for irradiation of the nanomaterial-based sensors by UV light. The closed irradiation chamber with the UV lamp integrated in the lid is shown in the picture on the right. The assembly of the sensor surface between the glass bottom substrate and the PDMS top substrate is in the upper left picture.

B. UV Irradiation Setup

The sensor surfaces were irradiated with a low-pressure UV lamp (8 W, multi-wavelength handheld lamp 3UV-38, UVP LLC, USA) at fixed wavelength of 254 nm. For reproducible results, the samples were irradiated in a custom-made closed aluminum-coated chamber of dimensions 50 cm × 17 cm × 20 cm (length × width × height) having the UV lamp integrated in the lid (Fig. 1). Specimens were irradiated at a fixed lamp-to-sample distance of 11 cm.

C. Characterization Methods

Contact angles were measured by the sessile drop method using a DataPhysics OCA15Pro analyzer system (DataPhysics Instruments, Germany) with ultrapure water as testing liquid or using the GNP/DNA dispersion. A minimum of five different drops (volume 3 µl) were analyzed for each substrate.

Electrical impedance spectroscopy in the frequency range from 20 Hz to 2 MHz was carried out using a precision E4980A LCR Meter (Agilent Technologies, USA). Measurements of the complex impedance Z were obtained at room temperature, and by placing the sensors inside a custom-built Faraday cage to protect the system from undesired noise. Conductive copper tape 0.035-mm thick (AT526-35 from Advance Tapes International, UK) was used to connect the sensor surfaces to the LCR meter.

III. Results and Discussion

A. Assembly of GNP and GNP/DNA Sensor Surfaces

Several UV sensor surfaces containing the hybrid GNP/dsDNA nanomaterials were prepared by evaporating aliquots (volume 1 mL) of aqueous dispersions in oven at 50 °C for 24 hours. Substrates made of glass and elastomeric PDMS were tested as biosensor supports. The efficiency of the double stranded DNA in dispersing the graphene nanoplatelets was assessed by analyzing under a microscope the homogeneity of the evaporated films (Fig. 2). Different dispersions were prepared by varying the ratio (by weight) of GNP and DNA. Similarly to previous investigations on the efficiency of DNA in dispersing carbon nanotubes in water [11, 13], we observed that an optimum dispersing efficiency is obtained by mixing GNP and DNA in a 1:1 ratio by weight (Fig. 2).

The role of the substrate on the properties of the nanostructured UV sensors was investigated by measuring the static contact angle of droplets of GNP and GNP/DNA aqueous solutions on glass and PDMS surfaces (Fig. 3). Generally, it was found that glass is a better substrate for these biosensors, in that the overall electrical resistance of the GNP/DNA films is lower on glass than on PDMS. This is due to the better adhesion and more homogeneous structure of the hybrid films on glass with respect to the elastomeric substrate. Furthermore, the flexibility of the PDMS support might induce micro-cracks in the GNP/DNA layer causing a decrease of its electrical properties.

Indeed, from the contact angle measurements we observed a much lower value of the contact angle of the GNP/DNA solutions on glass (28° ± 2°) than on PDMS surfaces (116° ± 3°) as shown in Fig. 3. As a consequence, evaporated layers of GNP/DNA are more homogeneous on the more hydrophilic glass, thus generating a biosensor surface with better electrical properties, and characterized by a more stable and reproducible signal as well.

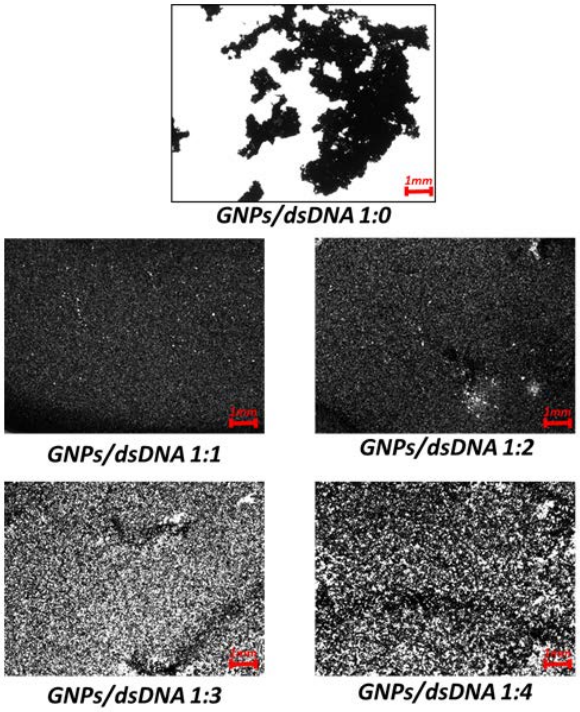

Figure 2. Optical images of nanostructured films containing the hybrid nanomaterial GNP/dsDNA at different weight ratios, showing the effect of the addtion of double stranded DNA to the homogenetity of GNP dispersions evaporated on the optically transparent surface of cell culture dishes. Scale bar is 1 mm.

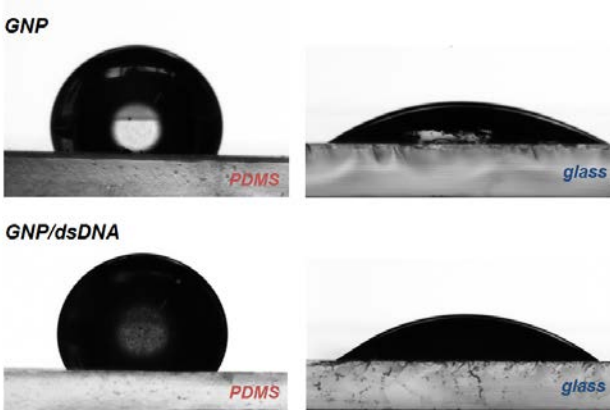

Figure 3. Contact angle images of aqueous droplets with GNP and GNP/dsDNA hybrids (drop volume 3 μl) on PDMS (*left*) and glass (*right*) substrates.

B. *Electrical Properties of GNP and GNP/DNA Sensors*

The electrical behavior of the sensor surfaces with different supports (glass and PDMS) investigated by impedance spectroscopy at frequencies ranging from 20 Hz to 2 MHz (Fig. 4). In both configurations of the sensor assembly, the sensing film was covered by PDMS substrate. PDMS was chosen as the material for the top substrate due to its transparency to UV light down to 240 nm [17], and therefore suitable to detect any effect of the 254-nm UV radiation on the underlying GNP/DNA films.

Fig. 4 shows the measured electrical impedance of the sensor surfaces for both configurations. The measured values refer to the impedance across the sensor surface. GNP and GNP/DNA films deposited on glass are characterized by much lower values of the electrical impedance in the entire frequency range 20 Hz – 2 MHz. On the other hand, films evaporated on PDMS surfaces show large values of the electrical impedance and high standard deviations at each frequency value. After these measurements, the sensors with glass bottom substrates showed overall higher conductivity and more stable properties, and therefore they were selected for further investigations by UV-C irradiation.

The electrical impedance response of the GNP/DNA sensor surfaces before and after irradiation by UV-C light was investigated by impedance spectroscopy in the frequency range of 20 Hz – 2 MHz. The electrical behavior was found to be purely resistive before and after irradiation of the surfaces. Therefore, we proceeded to measure only the electrical resistance at fixed time intervals over the course of irradiation using a handheld digital multimeter.

The behavior of the UV sensor surfaces GNP and GNP/DNA was distinctly different upon irradiation (Fig. 5). In general, sensor surfaces made of GNPs showed a significant increase of the electrical resistance upon irradiation, whereas film of the hybrid nanomaterial GNP/DNA had the opposite trend characterized by a decrease of the resistance over the course of the exposure to UV-C light. Such decrease of the resistance was of the order of 9% during the time of 8 hours for an exposed surface area of 0.5 cm^2. Several sets of samples (GNP and GNP/DNA) were

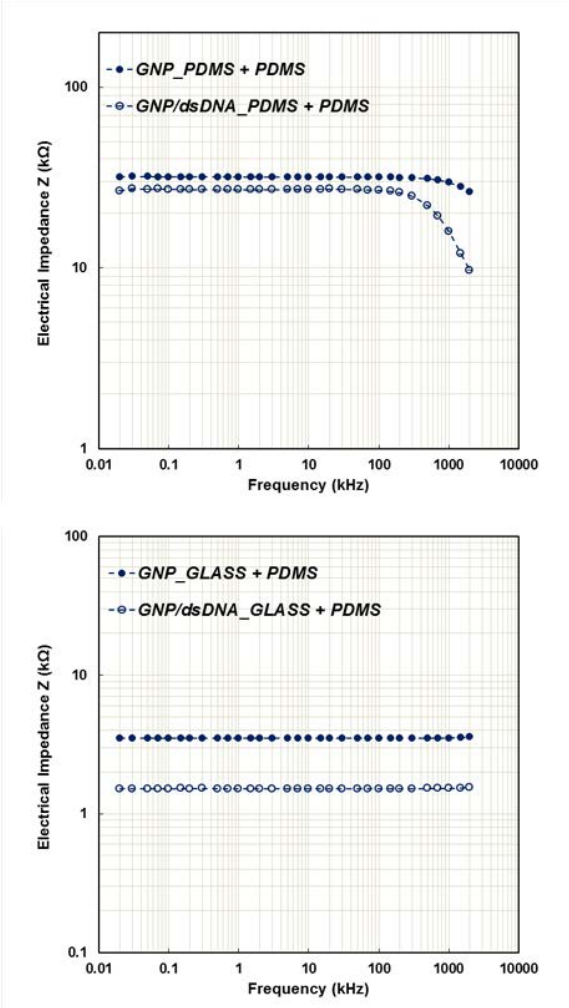

Figure 4. Measurements of the electrical impedance of the nanostructured films of GNP and GNP/dsDNA deposited on PDMS (top panel) and glass (bottom panel) surfaces. In both sensor configurations, the top cover is a PDMS substrate.

tested for data reproducibility. The response to UV-C irradiation was found to be consistent over all the analyzed samples.

IV. Conclusion

Sensor surfaces sensitive to irradiation by UV-C were prepared by depositing a thin layer of hybrid nanomaterial containing self-assembled GNP and double stranded DNA. The electrical properties of the GNP/DNA films deposited on glass bottom substrates and covered by PDMS top substrates vary in a consistent way upon exposure to UV-C light. In particular, The GNP/DNA films show a decrease of the electrical resistance, in contrast with the films made of GNP only where the resistance is observed to increase upon irradiation.

The results in this work are relevant for the design of novel sensing strategies for UV radiation, by which the biological damage to DNA can be directly assessed in real-time. Such sensors can find practical uses in all those environments where the biological effect of the damaging

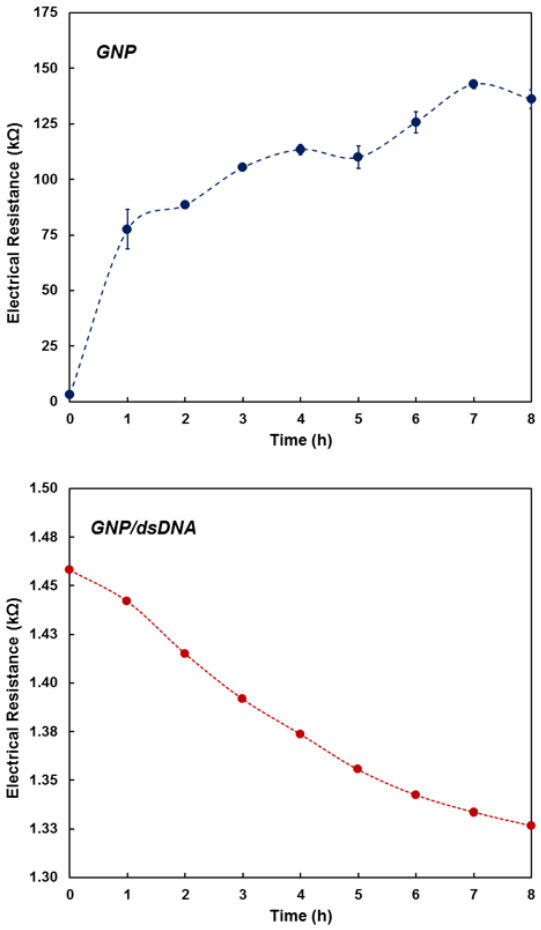

Figure 5. Variation of the electrical resistance of GNP and GNP/dsDNA sensor surfaces under irradiation by UV-C light for 8 hours. Sensors assembled using glass as bottom substrate and PDMS as top substrate. Each data point represents the average of five repeated measurements. Error bars refer to the standard deviation of the measurements.

UV-C radiation needs to be strictly monitored, both on Earth and in space environment.

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