Enhanced Sensing of Interleukin 8 by Stripping Voltammetry: Carbon Nanotubes versus Fullerene

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Abstract— **The ability to detect protein biomarkers at a sub-nanomolar level represents a pervasive challenge in order to bring a significant improvement in early diagnosis or progression of pato-physiological processes. To this aim, Screen Printed Electrochemical Sensors have been acquiring a predominant importance. The possibility to use them with different measurement techniques, and to customize their surface to improve the performance represent really attractive features. In this work, performances of two different carbon nanostructures in combination with Stripping Voltammetry were evaluated as tools to improve the detection of Interleukin 8, a cytokine that has pivotal roles in various inflammatory processes and considered as a universal biomarker. Commercially-available Carbon sensors were modified using Carbon Nanotubes and Spherical Fullerene through drop casting technique. Interleukin 8 was quantified using an indirect techniques based on silver stripping catalyzed using Alkaline Phosphatase. The nanostructured sensors showed better sensitivity with sub-nanomolar limit of detection: 0.39 ng/ml for carbon nanotubes and 0.61 ng/ml for fullerene compared to bare carbon electrodes. These modification method is promising for sensitive detection of protein biomarkers in several applications, including the monitoring of inflammatory processes.**

Keywords— **Interleukin 8, Stripping Voltammetry, carbon nanostructures, Fullerene, Carbon Nanotubes.**

I. Introduction

One of the most pervasive challenges in the field of pharmaceutical and medical research is the quantification of specific biomolecules inside biological fluids in the sub-nanomolar range in order to serve for early diagnosis of specific pathologies (e.g., cancer or neurodegenerative disease) [1] or for the effective and non-invasive monitoring of various physio-pathological processes (e.g., inflammation).

Nowadays the most commonly used technique for protein quantification is enzyme-linked immunosorbent assay (ELISA). Despite good limit of detection (pg/ml), it presents issues in terms of i) lack of standardization ii) high costs of implementation iii) impossibility to surface customization, preventing further improvements iv) impossibility to perform online monitoring. In order to overcome these limitations, Screen Printed Electrochemical Sensors (SPES) have attracted considerable interest in the last decade. Thanks to their low cost, ease of surface modification and of electrodes miniaturization SPES could represent a suitable solution to be integrated in complex devices (e.g. Lab on a Chip, Point of Care), thus obtaining fast, standardized and reliable electronic based protein quantifications, cost and time effective, maintaining the possibility to analyze samples (from standard solution to plasma) with an immunoenzymatic approach similar to ELISA [2].

In order to improve sensitivity, electrochemical measurement technique and surface design play a fundamental role.

Among the wide variety of electrochemical techniques, Stripping Voltammetry (SV) has been considered as one of the most promising one, in terms of both sensitivity and selectivity. Similar to other voltammetric methods, information about the analyte is obtained through current measurement over the scanned potential. More specifically, the analyte is pre-concentrated at the electrode and then stripped by application of a potential scan, thus increasing sensitivity up to 2 or 3 orders of magnitude, with a typical limit of detection (LOD) of 10^{-9} to 10^{-10} M [3]. Besides the applications to heavy metals quantification, interesting applications have been shown in the specific field of protein biomarkers [4].

In addition to the choice of the voltammetric techniques, the biosensor design strategies including nanostructuration of the sensor surface, via various materials to increase active surface area as well as electronic properties, have a big impact on ultra-sensitive sensor fabrication [5].

Among different types of materials, carbon nanostructures produced from graphite are promising low cost materials for biosensor design owing to their electronical performances and biocompatibility. Depending on the specific crystalline arrangement, they can be mainly classified as: spherical fullerenes (C_{60}), also referred as Buckminster-fullerenes, and cylindrical fullerenes, also known as carbon nanotubes (CNTs).

Both CNTs and C_{60} had a wide range of use as electrochemical biosensors due to their high surface to volume ratio and great electronical properties that enables high sensitivity and selectivity [6] [7]. Despite having the same bulk material, different arrangements of the stacked graphite layers produce

different behaviors in terms of increase in surface area and electronic properties [8].

As an important mediators of inflammation involved with pathogenesis of many inflammatory diseases, interleukin 8 (IL-8) is currently being used as non-invasive biomarker in various fields of medicine either for the purpose of early diagnosis or as a prognosis predictor [9]. Recently, Ray et al. [10] has identified IL-8 as a biomarker to diagnose and classify Alzheimer's disease. Therefore, IL-8 can be considered as a universal biomarker from cancer to inflammation [11] to neurodegeneration. Hence, the proposed work would have a widespread application from clinical diagnosis to more basic science studies like cell culture platforms such as organs-on-chips for monitoring cell secreted biomarkers for drug toxicity studies [12]. In the present work, SPES modified with above-mentioned nanomaterials were used to quantify IL-8 via SV. More specifically three different conditions (1. Bare Carbon, 2. Multi Walled CNTs (MWCNTs) modified Carbon and 3. C_{60} modified carbon) were biofunctionalized in the same manner to compare their performances in terms of LOD and sensitivity.

II. Materials and Methods

A. Materials

SPES (model DRP-C110) were purchased from DropSens (Spain). Electrodes consisted of a 0.12 cm^2 carbon working electrode, an Ag reference electrode and a carbon counter electrode. Powder ofMWCNTs (diameter: 10 nm; length: 1-2 m, 90 % purity, DropSens (Spain)) was dispersed in chloroform to the concentration of 2 mg/ml and the suspension was subjected to sonication for 1 h to achieve a homogeneous solution. Fullerene suspensions were obtained from Nanoshell (India). All the chemicals for electrolytic solutions were purchased from Sigma Aldrich. IL-8 quantification was performed using chemicals from a dedicated kit (DuoSet® for ELISA, Human CXCL8/IL-8).

B. Quantification of active surface area

The comparison between C_{60} and MWCNTs was done in terms of sensitivity, LOD and redox potentials taking the active surface area into account, since it is shown to be directly related to sensitivity (S) [13]. Cottrell and Randles Sevcik equations were used to calculate the surface area of electrodes due to its linear relationship with faradaic current. The total surface area (TSA) increase obtained with 20 µg of MWCNTs (calculated as 4314 mm^2) was considered as the reference [14]. In order to assure the same active surface area of SPES after nanostructure modification with C_{60}, specific surface area (SSA) of C_{60} was calculated by using eq. 1 as 29 m^2/g. The nanostructure modification was done via drop casting dispersion of C_{60}, with an average dimension of 120 nm. Thus, the amount of C_{60} needed for the modification was calculated as 149 µg by using the following eqs. 2 and 3,

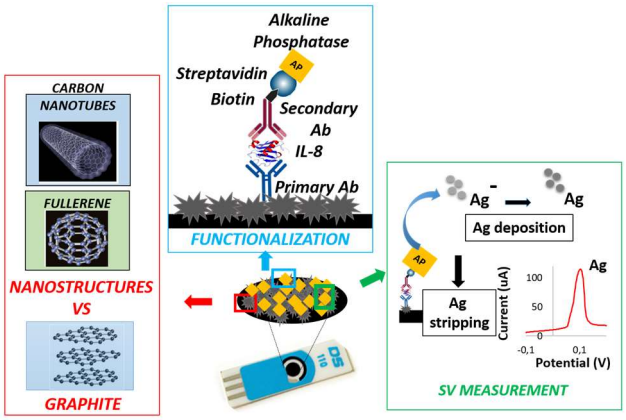

Figure 1. Schematics of the proposed nanostructured electrochemical sensor for IL-8 detection

$$SSA_{C60} = A/mass = A/V*\rho = 3/r*\rho \quad (1)$$

$$TSA_{ref} = TSA_{CNTs}\ (20ug) = SSA_{CNTs} * mass_{CNTs} \quad (2)$$

$$C_{60\ mass} = TSA_{ref} / SSA_{C60} \quad (3)$$

C. Preparation and characterization of electrodes

Nanostructure modified SPES were prepared by drop casting technique [14] as follows: for MWCNTs 10 µl of 2 µg/ml suspension and for C_{60} 149 µl of 1 µg/ml suspension were deposited, and allowed to dry after each deposition step. Electrodes were stored at room temperature after the deposition. Electrochemical Cyclic Voltammetry (CV) was performed under aerobic conditions using AutoLab potentiostat/galvanostat (Metrohm). Sensors were covered with 100 µl of 0.1 M KCl solution, then the device was configured to sweep at a scan rate of 100 mV/s in the range of -1 to +1 V.

In order to optimize Anodic SV (ASV) parameters, Ag-stripping in KCl solution was performed. Specifically, sensors were covered with 100 µl of a solution containing 1 mM of silver nitrate and the potential was set at -0.7 V for 20 s in order to allow Ag pre-concentration. After that, the solution was removed and a linear sweep voltammetry (LSV) was performed in KCl up to +0.5 V (vs Ag). All the experiments were performed in triplicate. Peaks height and position of voltammograms were assessed by curve fitting using a dedicated analysis tool (Nova 1.11).

D. SPES bio-functionalization

All electrodes were exposed to the same bio-functionalization steps as following:

i) 2 h immobilization of IL-8 antibody to sensor surfaces via drop-casting, ii) 2 h incubation with IL-8 samples, iii) 1 h 30 min incubation with biotin-labelled detection antibody iv) 30 min addition of streptavidine-tagged Alkaline Phosphatase (AP) enzyme that catalyzes the oxidation of ionic Ag ($AgNO_3$) to metallic Ag, thanks to the reaction happening in presence of Ascorbic acid (AA-p), as described in [15] (Fig.1). For every step, 20 µl of solution were drop-casted on the WE.

E. IL-8 quantification using ASV

Once the bio-functionalization was completed, sensors were covered with 100 µl of 0.1 M KCl, and constant potential of -0.12 V was applied for 5 s and then LSV performed at a scan rate of 40 mV/s up to +0.4 V, measuring Ag oxidation current. Due to Ag deposition, each concentration was quantified on single-use disposable SPES. The first calibration of the three conditions was performed in the range of IL-8 concentration from 5 ng/ml and 20 ng/ml, to evaluate the specific LOD for each condition (bare SPE, C_{60} modified SPE, MWCNT modified SPE). A second calibration considering lower levels of concentrations (1.25-5 ng/ml) was performed as well in order to compare nanostructured sensors.

III. Results

A. Electrodes preparation and characterization

Characterization of nanostructures via CV in 0.1 M KCl showed an enhanced response given by MWCNTs modified electrodes in term of peak height, while an enhanced response in term of capacitive current was observed for C_{60} modified electrodes (Fig.2).

Ag peaks could be observed in all the conditions showing a shift of 100 mV toward higher values of potential for both MWCNTs and C compared to bare carbon. In term of peak height, MWCNTs showed the most enhanced behavior (Carbon 144 ± 16 µA; MWCNTs 208 ± 12 µA; C_{60} 168 ± 63 µA).

B. IL-8 quantification using ASV

In all the calibrations, nanostructured electrodes showed an enhanced response in terms of sensitivity and LOD.
Specifically, in the range between 5 and 20 ng/ml, C_{60} showed a higher response compared to MWCNTs. LOD was calculated to be 4.41 ± 0.88 ng/ml, 0.84 ± 0.18 ng/ml and 0.67 ± 0.05 ng/ml for bare SPES, MWCNTs and C_{60} respectively, showing the effect of nanostructures in enhancing sensitivity of the sensors.
On the other hand, in the range of lower levels of concentrations, MWCNTs (LOD = 0.39 ± 0.10 ng/ml) performance appears to be superior compared to C_{60} (LOD= 0.61 ± 0.05 ng/ml) (Fig.3). In term of peak positions, in all the three types of electrode modifications, Ag peaks shifted to more positive potentials by increasing the concentration of IL-8 as a known phenomenon in voltammetry.

IV. Discussion

The gain introduced by the use of nanostructures in terms of additional electroactive surface area and effects of different materials dealing with the same increase of surface area have been previously demonstrated [13].

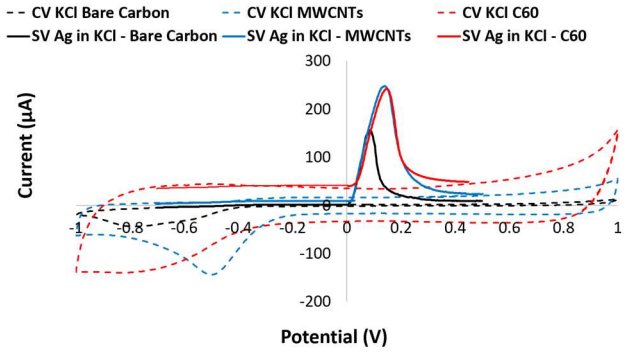

Figure 2 Electrochemical characterization of nanostructured electrodes via CV (dashed) and Ag stripping via LSV (continuous),both in 0.1 M KCl

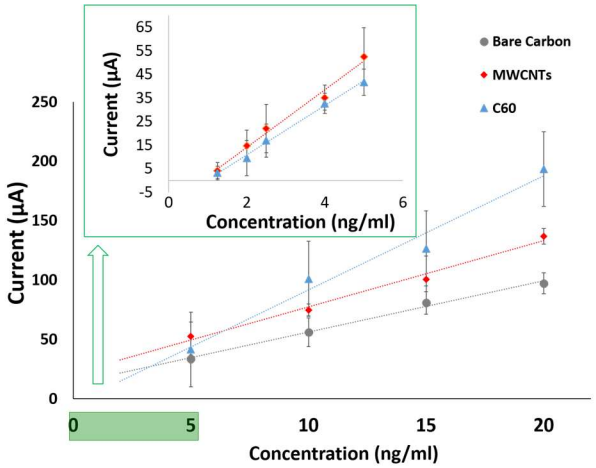

Figure 3 Calibration of the 3 electrodes in the range 5-20 ng/ml and comparison between MWCNTs and C60 performances in the range 1.25 – 5 ng

The enhancement in the electrochemical response we obtained by introducing nanostructures in CV experiments performed in KCl is in good agreement with the literature [16]. Despite the same bulk materials, significant differences could be observed between different nanostructures suggesting that other phenomena are in place when dealing with differently arranged nanostructures (e.g. porosity, surface pattern, electronical properties). Results from SV of Ag in KCl suggested that the selected parameters are optimal to detect Ag peak and that KCl represents a suitable supporting buffer due to absence of redox peaks near to Ag oxidation potential for all the structured electrodes.

Difficulties in obtaining a reliable LOD < 5 ng/ml by using bare carbon SPES have been previously reported by several groups [4]. However, the combination of nanostructures and stripping voltammetry appears to be really promising for a sensitive detection of IL-8 as biomarkers. Our work proves that nanostructuration can increase the LOD up to 8 fold compared to bare electrodes, [17]. More specifically, the enhanced response of C_{60} with higher concentrations is attributed to its particular zero-dimensional structure, as highlighted by Han et al [18]. The fullerene C_{60} has been widely investigated as the optimal solution to provide effective coating with peptides, antibodies, amino acids and various other molecules. Its ability to maximize the performance of the sensor is usually registered in an improvement of the sensitivity and LOD, especially for higher values of concentration. The lower LOD showed by MWCNTs for lower concentrations is explained taking into account the superior electron-transfer properties of MWCNTs over C_{60} which contribute in enhancing the signal for even very low amounts of the analyte [19].

Results obtained with both MWCNT and C_{60} nanostructures seem to be very promising, especially for monitoring inflammation processes that typically have IL-8 in the range of 1-10 ng/ml [11]. Furthermore, the low variability observed, the possibility to optimize biomolecules coating and to miniaturize the electrodes, suggest the possibility to reach LOD actually quantifiable with ELISA (pg/ml), with higher reliability and standardization, lower costs of implementation, both in term of time and volume of samples required.

V. Conclusion

Herein by this work, IL-8 quantification with a sub-nanomolar LOD (<1 ng/ml) and high repeatability was achieved thanks to MWCNT and C_{60} nanostructures and stripping voltammetry. Designed sensors are very promising candidates for advanced integrated point-of-care or organ-on-chip monitoring systems. Future work will focus on investigations of the interactions between nanostructures and biomolecules, via different nanostructuration methods of SPES.

Conflict of Interest

The authors declare that they have no conflict of interest

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References

[1] N. Lu *et al.*, "Ultrasensitive Detection of Dual Cancer Biomarkers with Integrated CMOS-Compatible Nanowire Arrays," *Anal. Chem.*, vol. 87, no. 22, pp. 11203–11208, Nov. 2015.
[2] R. A. S. Couto, J. L. F. C. Lima et al., "Recent developments, characteristics and potential applications of screen-printed electrodes in pharmaceutical and biological analysis," *Talanta*, vol. 146, pp. 801–814, Jan. 2016.
[3] J. Barón-Jaimez, M. R. Joya et al., "Anodic stripping voltammetry – ASV for determination of heavy metals," *J. Phys. Conf. Ser.*, vol. 466, p. 12023, 2013.
[4] V. Escamilla-Gómez, D. Hernández-Santos et al., "Simultaneous detection of free and total prostate specific antigen on a screen-printed electrochemical dual sensor," *Biosens. Bioelectron.*, vol. 24, pp. 2678–2683, 2009.
[5] L. Syedmoradi, M. Daneshpour et al., "Point of care testing: The impact of nanotechnology," *Biosens. Bioelectron.*, vol. 87, pp. 373–387, 2017.
[6] S. Afreen, K. Muthoosamy et al., "Functionalized fullerene (C60) as a potential nanomediator in the fabrication of highly sensitive biosensors," *Biosens. Bioelectron.*, vol. 63, pp. 354–364, 2015.
[7] C. Boero, S. Carrara et al., "Highly sensitive carbon nanotube-based sensing for lactate and glucose monitoring in cell culture.," *IEEE Trans. Nanobioscience*, vol. 10, no. 1, pp. 59–67, Mar. 2011.
[8] S. Cinti *et al.*, "Screen-Printed Electrodes Modified with Carbon Nanomaterials: A Comparison among Carbon Black, Carbon Nanotubes and Graphene," *Electroanalysis*, vol. 27, no. August, pp. 2230–2238, 2015.
[9] A. Shahzad, M. Knapp et al., "Interleukin 8 (IL-8) - a universal biomarker?," *Int. Arch. Med.*, vol. 3, p. 11, Jun. 2010.
[10] S. Ray *et al.*, "Classification and prediction of clinical Alzheimer's diagnosis based on plasma signaling proteins," *Nat Med*, vol. 13, no. 11, pp. 1359–1362, Nov. 2007.
[11] H. J. Kim, H. Li et al., "Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip," *Pnas*, no. 13, pp. E7–E15, 2015.
[12] S. R. Shin *et al.*, "Aptamer-Based Microfluidic Electrochemical Biosensor for Monitoring Cell-Secreted Trace Cardiac Biomarkers," *Anal. Chem.*, vol. 88, no. 20, pp. 10019–10027, 2016.
[13] C. S. Aliakbarinodehi Nima, Taurino Irene et al., "Electrochemical nanostructured biosensors: carbon nanotubes versus conductive and semi-conductive nanoparticles," *Chem. Pap.*, vol. 69, p. 134, 2015.
[14] N. Aliakbarinodehi, G. De Micheli et al., "Optimized electrochemical detection of anti-cancer drug by carbon nanotubes or gold nanoparticles," *2015 11th Conf. Ph.D. Res. Microelectron. Electron. PRIME 2015*, pp. 25–28, 2015.
[15] Z. P. Chen, Z. F. Peng et al., "An electrochemical amplification immunoassay using biocatalytic metal deposition coupled with anodic stripping voltammetric detection," *Sensors Actuators, B Chem.*, vol. 129, pp. 146–151, 2008.

[16] E. Jubete, O. a. Loaiza et al., "Nanotechnology: A Tool for Improved Performance on Electrochemical Screen-Printed (Bio)Sensors," *J. Sensors*, vol. 2009, 2009.
[17] C. Yang, M. E. Denno et al., "Recent trends in carbon nanomaterial-based electrochemical sensors for biomolecules: A review," *Anal. Chim. Acta*, vol. 887, pp. 17–37, Aug. 2015.
[18] X. Han *et al.*, "Interactions between Carbon Nanomaterials and Biomolecules," *J. Oleo Sci.*, vol. 65, no. 1, pp. 1–7, 2016.
[19] S. N. Kim, J. F. Rusling et al., "Carbon Nanotubes for Electronic and Electrochemical Detection of Biomolecules," *Adv. Mater.*, vol. 19, no. 20, pp. 3214–3228, Oct. 2007.

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