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COMMUNICATION

Glass/PDMS hybrid microfluidic device integrating vertically aligned SWCNTs to ultrasensitive electrochemical determinations†

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This communication reports a promising platform for rapid, simple, direct, and ultrasensitive determination of serotonin. The method is related to integration of vertically aligned single-walled carbon nanotubes (SWCNTs) in electrochemical microfluidic devices. The required microfabrication protocol is simple and fast. In addition, the nanomaterial influenced remarkably the obtained limit-of-detection (LOD) values. Our system achieved a LOD of 0.2 nmol L^{-1} for serotonin, to the best of our knowledge one of the lowest values reported in the literature.

Glass/elastomer hybrid microfluidic platforms with integrated electrochemical detectors are powerful analytical tools. Their main advantages include: (*i*) low cost; (*ii*) chemical inertia; (*iii*) optical transparency; (*iv*) thermal, mechanical, and chemical stability; (*v*) automation capacity, and; (*vi*) easy microfabrication. In addition, they offer high values of reproducibility and sensitivity.[1–3] Glass is widely used for the deposition of metallic thin films, which act as electrodes.[4] Poly(dimethylsiloxane) (PDMS), in turn, is the elastomer most frequently used to fabricate microstructures. PDMS has a high heat distortion temperature (200 °C) and low surface energy (20 erg cm^{-2}), making it the elastomer of choice for microfabrication processes. This polymer can be easily patterned *via* soft lithography and sealed against many flat surfaces, reversibly or irreversibly, facilitating the integration of valves, pumps, reactors, and detectors in chips.[5] Additionally, transparency and gas permeability are useful properties for cell culture assays. Finally, the adsorption/absorption of hydrophobic small molecules onto/into the porous structure of the PDMS allows its use in microextraction techniques. Nevertheless, this phenomenon can change the concentration of analyte in the sample and the experimental outcomes at other applications. Some methods that aim to reduce the effects of the adsorption and absorption in PDMS channels can be found in the literature.[6,7]

Recent papers reported the use of non-aligned carbon nanotubes (CNTs), multi- (MWCNTs) and single-walled (SWCNTs) carbon nanotubes, in electrochemical microdevices for flow analysis.[8–13] This platform was applied also in a capillary electrophoresis (CE) microchip.[14,15] Applications in electrophoretic systems, meanwhile, exhibit drawbacks related to interferences between the electric fields of detection and separation.[4] CNT is a nanomaterial broadly used for the development of detectors and sensors.[16–18] Such compounds aggregate advantages which can improve the selectivity and sensitivity of the analytical method. Their main characteristics include: (*i*) high area/volume ratio (electrocatalytic activity); (*ii*) good thermal and chemical stabilities; (*iii*) conducting (ideal for nanoscale electrodes)[19] or semiconducting (excellent for nanoscale field effect transistors)[20] behavior; and (*iv*) high surface area.[18] The performance of electrochemical CE microchips with different electrode substrates covered by MWCNTs and SWCNTs was evaluated by Pumera *et al.*[21] CNTs-modified electrodes displayed the best signal/noise (S/N) ratio and resolution when compared with a bare electrode, reflecting the electrocatalytic power and the increase of the surface area by this nanomaterial.

A potential alternative in improving the electronic characteristics of the CNTs is the use of vertically aligned carbon nanotubes using single-stranded DNA (ssDNA) as an anchor group. Different articles described procedures for alignment of CNTs; these reports showed that the detectors/sensors integrating aligned CNTs exhibit fast electronic transfer, electrocatalysis, and high sensitivity in electrochemical assays.[22,23]

This communication describes a rapid, simple, portable, direct, and ultrasensitive approach to electrochemical determination of serotonin in a glass/PDMS microfluidic device with vertically aligned SWCNTs. Serotonin is the major neurotransmitter in the brain and alterations of its levels in humans can be related to some diseases such as Alzheimer's, infantile autism, sleeping disorders, depression, and bulimia.[24,25] Different techniques have been used to quantify this neurotransmitter, including indirect (LC-MS and CE-LIF) and direct methods, which consist of electrochemical sensors/detectors.[24–29] The main interferents for serotonin in electrochemical analysis are dopamine, ascorbic acid, and uric acid. In this context, CNTs provide many active sites that enhance the sensitivity and resolution (in terms of oxidation potential) in voltammetry. Thus, it becomes unnecessary to modify the surface

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of CNTs, to pre-treat the sample, or to use separation techniques for specific determinations of serotonin in complex matrices such as biological samples.[29] Considering these implications, we developed a SWCNTs-based voltammetric system in order to achieve a high-throughput tool for serotonin assay. Additionally, the method contemplates inherent advantages associated with microfluidic, hybrid microchips, and vertically aligned SWCNTs.

The fabrication of the microdevices involved the following main steps: (*i*) sputtering deposition of the electrodes (Ti/Au) on a flat glass bottom, (*ii*) molding of the PDMS microchannels by soft lithography, (*iii*) sealing of the glass/PDMS device irreversibly, and (*iv*) immobilizing of the SWCNTs *in situ* over a working electrode. More details about steps (*i*)–(*iii*) can be found in ref. 30 and 31. Fig. 1 illustrates the microchip layers (Fig. 1a) and a photo of the resulting device (Fig. 1b), showing the Au thin films functioning as working (WE), counter (CE), and pseudo-reference electrodes (RE). The microfluidic channels were shaped in a Y-configuration (two inlets and one outlet) with 250 μm width and 50 μm height, whereas the electrodes presented 1.0 mm of width and spacing.

The SWCNTs immobilization was carried out as follows. Prior to the modification of the Au surface, 100 mg SWCNTs (98% purity, NanoLab, Waltham, MA, USA) synthesized by thermal chemical vapor deposition were mixed with 100 mL HNO_3/H_2SO_4 (3 : 1, v/v) solution for 12 h aiming to promote the CNTs functionalization.[32] The SWCNTs were then filtered through a Millipore Nylon® filter membrane. The resulting nanotubes were continuously washed using distilled water until the pH of the filtrate was neutral. In the sequence, SWCNTs were dried overnight in a vacuum oven at 120 °C. The formation of the ssDNA/SWCNT hybrid on Au surface was based on the work of Zheng *et al.*[33] (thiol-terminated ssDNA with the following sequence 5-HS-TGG-GGT-TTA-TGG-AAA-TTG-GAA-3′ was purchased from SinapseBiotecnologia, São Paulo, Brazil). First, ssDNA wraps the CNTs by π–π interactions, which involve aromatic rings of the ssDNA molecule nucleotide

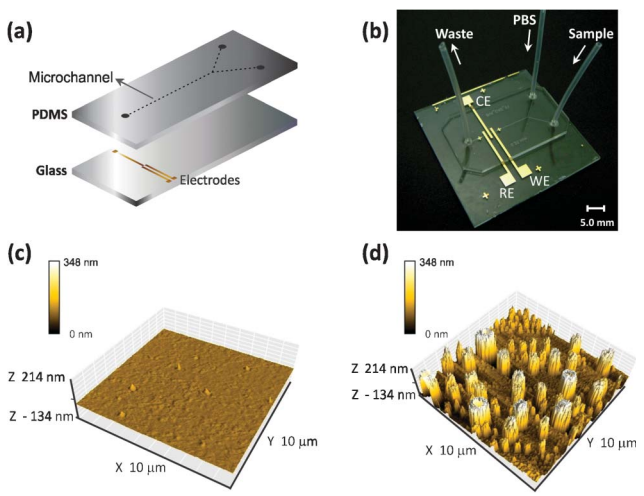

Fig. 1 Microfluidic device and morphologic characterization of the working electrode. Schematic diagram of the layers that compose the microchip (a), photo of the device (b), and AFM images of the unmodified Au electrode (c) and Au modified with SWCNTs surfaces (d). WE, CE, and RE are working (containing the SWCNTs), counter, and pseudo-reference electrodes, respectively, and PBS is phosphate buffer solution.

bases and the hydrophobic side of CNTs; the hydrophilic (sugar-phosphate) of ssDNA is left exposed to interactions with the medium. The formed ssDNA/SWCNTs hybrid is then assembled on the Au surface by spontaneous adsorption of thiol groups. For this, 1.0 mg functionalized SWCNTs was mixed with 500 μL of 1.0 μmol L^{-1} ssDNA solution. The latter was prepared in 0.1 mol L^{-1} phosphate buffer solution (PBS) containing 10% sodium chloride (v/v). Next, the mixture was sonicated using an ultrasonic horn probe and then centrifuged at 7000 rpm; each process took 45 min. Finally, the supernatant solution was collected and then pumped into the microfluidic channel until it reached the detection zone on WE (Fig. 1b). Self-assembled monolayers (SAMs) consisting of ssDNA/SWCNT were formed after 24 h in a refrigerated room at 4 °C.

The morphological characteristics of the electrodes were examined by atomic force microscopy (AFM) using a model Nanosurf EasyScan 2 AFM System (Nanosurf AG, Switzerland). The measurements were carried out with non-contact/tapping mode, long cantilever. Fig. 1c displays a typical image of the gold surface, which was shown to be flat and smooth. Based on Au surface topography analyses, the root mean square (RMS) roughness was 1.5 nm. Fig. 1d shows the WE after alignment procedure of the SWCNTs. In this image, we observed that the SWCNTs were vertically aligned and bundled. The RMS roughness was 55 nm whereas the SWCNTs average height was equal to 123.9 nm with relative standard deviation (RSD) of 11.8%.

Flow analyses were carried out in order to assess the electrochemical behaviour of the serotonin, study the interference level of the ascorbic (AA) and uric (UA) acids, and quantify serotonin standards by chronoamperometry. The device has two inlets and one outlet (for waste) vias as shown in Fig. 1b. Measurements of the current were performed in real time while either buffer or analyte solution was flowing through the microfluidic channels (in cyclic voltammetry (CV) the measurements were performed under stationary flow). Two external syringe pumps model NE-300 (New Era Pump Systems Inc., Farmingdale, NY, USA) were used.

The electrochemical behavior of serotonin at the Au/ssDNA/SWCNT electrode was evaluated in solution containing 0.1 mol L^{-1} PBS (pH 6.0) and 100 μmol L^{-1} serotonin. The experiments were carried out by CV using a scan rate of 50 mV s^{-1} and differential pulse voltammetry (DPV), with an amplitude of 100 mV and a step potential equal to 2 mV. All electrochemical measurements were performed using a model PGSTAT 30 Autolab electrochemical system (Eco Chemie, Utrecht, Netherlands) equipped with PGSTAT-12 and GPES software (Eco Chemie). The results are presented in Fig. 2.

As verified in Fig. 2a, the Au/ssDNA/SWCNT electrode exhibited a well-defined oxidation peak at a potential value of +400 mV. This peak is attributed to an irreversible oxidation of the hydroxyl group present in the aromatic ring of the serotonin forming ketone species.[34] The Au/ssDNA/SWCNT surface caused a shift of 140 mV in the oxidation potential of serotonin to a less positive value when compared to the bare Au electrode (+540 mV). Additionally, the oxidation on such a catalytic surface showed a 3-fold increase in peak current compared to the unmodified one. The shift in the potential value and the increase in the current peak reflect, respectively, the electrocatalytic activity and the increase of the electroactive surface area by the aligned

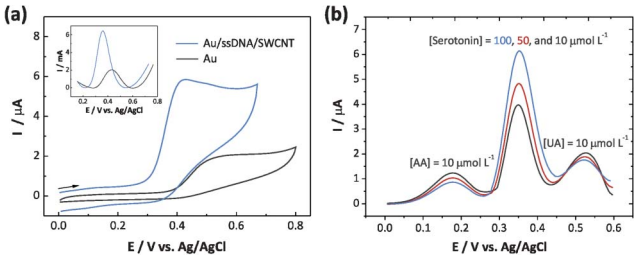

Fig. 2 Electrochemical characterization of the system (a) and study of interferents (b). CV and DPV (inset) experiments for Au (gray lines) and Au/ssDNA/SWCNT (blue lines) electrodes in solution containing 0.1 mol L^{-1} PBS (pH 6.0) and 100 μmol L^{-1} serotonin (a). DPV experiments for Au/ssDNA/SWCNT electrodes in solution with 0.1 mol L^{-1} PBS (pH 6.0), concentrations of ascorbic acid (AA) and uric acid (UA) fixed at 10 μmol L^{-1}, and sequential additions of serotonin standards in the following concentrations: 10, 50, and 100 μmol L^{-1} (b). Conditions: 50 mV s^{-1} scan rate (CV), 100 mV amplitude (DPV), 2 mV step potential (DPV).

SWCNTs. Such properties make the Au/ssDNA/SWCNT hybrid a promising setup for serotonin detection as discussed below.

The interference of AA and UA is one of the main problems in the electrochemical determination of serotonin in biological fluids. In this sense, the significance of the interference of these species in the electrooxidation process of the serotonin on the Au/ssDNA/SWCNT surface was evaluated. The experiments were carried out using DPV with optimized parameters (see below) in solutions with 0.1 mol L^{-1} of PBS (pH 6.0), concentrations of AA and UA fixed at 10 μmol L^{-1}, and sequential additions of 10, 50, and 100 μmol L^{-1} serotonin. Fig. 2b shows some of the results. It can be noted that, even at equal concentrations of AA, UA, and serotonin, there is no overlap of the serotonin oxidation peak with AA and UA peaks in the voltammetric profile. Instead, with the increase in the concentration of serotonin, the procedure yielded totally separated responses for all species, indicating thus that the Au/ssDNA/SWCNT electrode is adequate to be used for selective determination of serotonin in the presence of AA and UA.

Before the chronoamperometric determination of serotonin standards, some experimental parameters (sample and carrier flow rate and potential) were optimized. The influence of the applied potential in the amperometric detection was investigated in order to maximize the analytical signal and to improve the sensitivity of the method. The following working potential values were investigated: +0.2, +0.3, +0.4, +0.5, and +0.6 V. The transient signal of the current has a maximum value at +0.4 V indicating the best operating potential; therefore +0.4 V was chosen as the working potential for the subsequent studies. The effect of the sample flow rate, ranging from 5.0 to 30.0 μL min^{-1} on the analytical signal, was evaluated by injecting (5 s) 100 μmol L^{-1} serotonin solution in 0.1 mol L^{-1} PBS (pH 6.0). The flow rate of the carrier (0.1 mol L^{-1} PBS, pH 6.0) was fixed at 5.0 μL min^{-1}. The transient signal of the current increased with the sample flow rate in the studied range so that the optimal flow rate was of 30.0 μL min^{-1}. Using the same solution and injection time, the effect of the carrier flow rate was also investigated in a range from 5.0–30.0 μL min^{-1} with the sample flow rate kept constant at 5.0 μL min^{-1}. The current decreased with increase of the carrier flow rate in the studied range. Thus, the optimal carrier flow rate was 5.0 μL min^{-1}.

Using the optimized conditions as previously described, chronoamperometry was used to investigate the electrochemical response as a function of the analyte concentration. In this study, microfluidic devices containing Au and Au/ssDNA/CNTs as the working electrodes were employed. Serotonin standards were used for construction of the calibration curves as shown in Fig. 3a. We performed three measurements for each concentration level. Fig. 3b presents the signals recorded for the Au/ssDNA/CNTs electrode, which correspond to the current peak of serotonin oxidation.

The analytical signals exhibited a linearity range from the limits-of-detection (LOD) up to 10.0 μmol L^{-1} for both Au (R^2 = 0.9969) and Au/ssDNA/SWCNT (R^2 = 0.9991) electrodes. The detectability levels were remarkably influenced by the use of SWCNTs: LODs equal to 11.8 (Au) and 0.2 nmol L^{-1} (Au/ssDNA/SWCNTs) were determined using 3σ/slope ratio (σ is the standard deviation of the mean current for 10 chronoamperograms of the blank). The calculations proceeded according to the recommendations of IUPAC.[35] The 0.2 nmol L^{-1} LOD is 25 times lower than previous values reported in the literature (5.0 nmol L^{-1}), obtained by a SWCNT-modified glassy carbon electrode.[29] The effect of the aligned SWCNTs can be further evaluated from the values of the analytical sensitivity, which were almost three times higher with Au/ssDNA/SWCNT (4.54 nA μmol^{-1} L) than that obtained with the unmodified Au surface (1.33 nA μmol^{-1} L).

Serotonin has been determined by several techniques, which integrate separation methods—such as liquid chromatography[36–38] and capillary electrophoresis[39]—or direct assays using potentiometry[27,29,40] and sensor-based applications.[41,42] In general, as verified in Table 1, our set up shows a LOD lower than those reported in the literature even when a preconcentration step was employed.

The inter-assay precision (microfabrication reproducibility) of the Au/ssDNA/SWCNTs microdevice was measured from five experiments on different days. Prior to each experiment, the electrode surfaces were rinsed thoroughly with double-distilled water. Then, seven injections of 100 μmol L^{-1} serotonin in 0.1 mol L^{-1} PBS (pH 6.0) were performed for each microchip. The RSD was calculated as 2.3%. In addition, intra-assay precision tests were performed from ten injections of that same solution. The RSD was found to be 1.7%. Finally, the analytical frequency of the

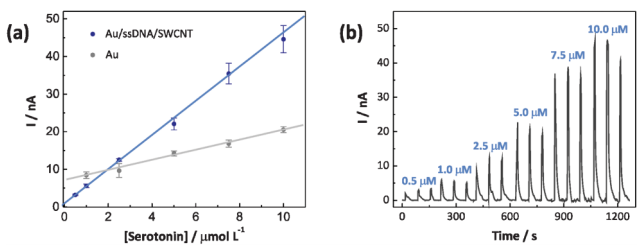

Fig. 3 Chronoamperometric determinations of serotonin standards. Analytical calibration curves obtained in microfluidic devices integrating the Au (gray circles) and Au/ssDNA/CNTs (blue circles) electrodes (a) and signals associated with the Au/ssDNA/CNTs electrode (b). Conditions: solution containing 0.1 mol L^{-1} PBS (pH 6.0) and serotonin standards. Potential, injection time, and sample-carrier flow rate are the same as described in Fig. 2.

Table 1 Comparison of a few reported values for LOD of serotonin

Detector	LOD (nmol L^{-1})	Reference
Potentiometry (using MWCNT-modified glassy carbon electrode)	500	27
Potentiometry (using SWCNT-modified glassy carbon electrode)	100	29
HPLC-mass spectrometry	18.2	37
CE with microwave extraction, sample stacking, and preconcentration	7.9	39
Potentiometry (using modified indium tin oxide electrode)	0.1	40
Potentiometry (using Au/polypyrrole-modified glassy carbon electrode)	1.0	41
Electrochemical sensor (using cyclodextrin)	200	42
Microfluidic device with aligned SWCNTs	0.2	This work

Au/ssDNA/SWCNTs microfluidic device for serotonin detection was calculated in 55 determinations per hour.

The use of vertically aligned SWCNTs in an electrochemical microfluidic device demonstrated to be a promising alternative to improve electrochemical determination of important biomarkers such as serotonin. Regarding the use of Au as the working electrode in chronoamperometry measurements, the Au/ssDNA/SWCNTs system improved the analytical sensitivity almost three fold. There are a number of advantages in using glass/elastomer microfluidic platforms with electrochemical detectors, including: (*i*) low cost; (*ii*) easy microfabrication; (*iii*) automation capacity; (*iv*) good analytical frequency; (*v*) reproducibility, and; (*vi*) increased sensitivity.

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