Stochastic Microsensors

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Two three-dimensional (3D) stochastic microsensors based on immobilization of protoporphyrin IX (PIX) in single-walled carbon nanotubes (SWCNT) and multi-walled carbon nanotubes (MWCNT) decorated with copper (Cu) and gold (Au) nanoparticles were designed and used for the molecular recognition of isocitrate dehydrogenase 1 (IDH1) and isocitrate dehydrogenase 2 (IDH2) in biological samples (brain tumor tissues, whole blood).

stochastic microsensors | isocitrate dehydrogenase 1 | isocitrate dehydrogenase 2

1. Introduction

Enzyme and gene assays play a very important role in cancer diagnosis. There are two genes—human isocitrate dehydrogenase (IDH) isoforms—which are homodimer isoenzymes: IDH1 found in cytoplasm and peroxisomes, and IDH2 in mitochondria. IDH1 and IDH2 play a very important role in the diagnosis of brain cancer [1][2][3][4][5][6]. Accordingly, they can be used as biomarkers for the rapid diagnosis of brain cancer/gliomas, which are encountered frequently in highly developed countries and have the worst prognosis among solid cancers. Diffuse gliomas are the most common primary brain tumors found in adults, affecting approximately 20,000 people annually in the United States [7].

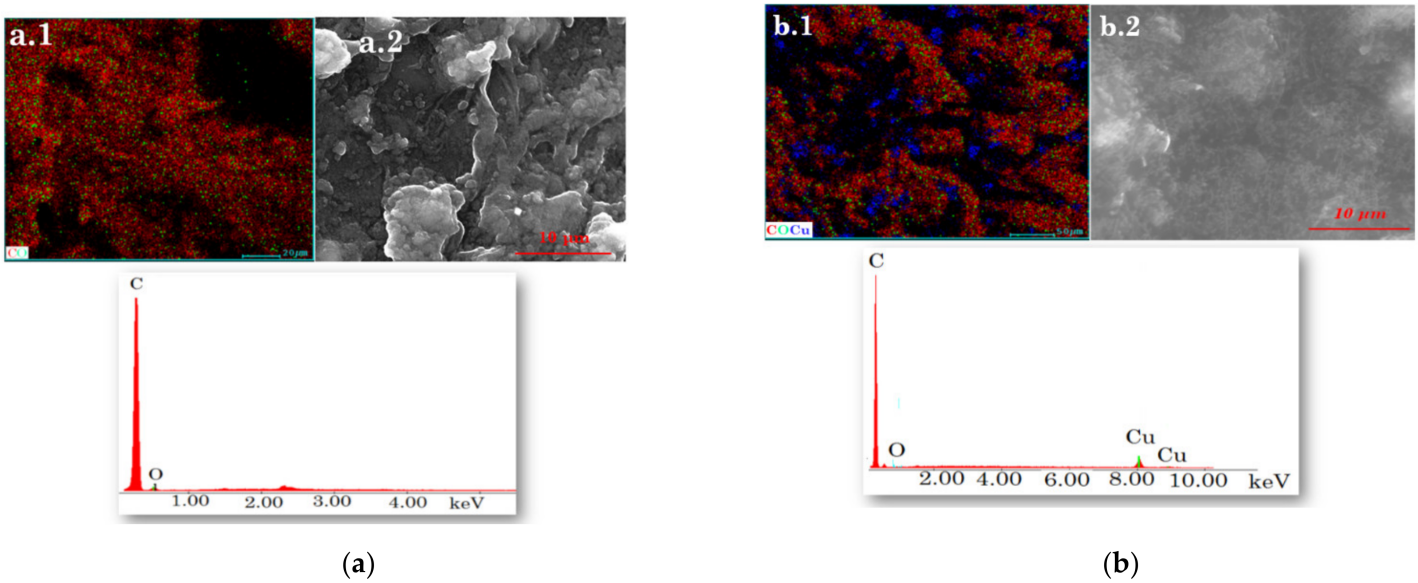
To date, polymerase chain reaction (PCR) and deoxyribonucleic acid (DNA) sequencing are the main techniques used for the assay of IDH1 and IDH2, e.g., DNA pyrosequencing was proposed for the assay of IDH1 and IDH2 [8]. A multiplex-based bead assay [9] and a fluorescence method [10] were also proposed for the assay of IDH1 and IDH2. The only sensors proposed to date are the 2D disposable stochastic sensors, which are capable of determining IDH1 and IDH2 in whole blood and tissue samples [11]. There are numerous commercial ELISA kits used for the assay of IDH1 and IDH2 in clinical laboratories as standard methods. The US Food and Drug and Administration (FDA) office recently approved a method for the assay of IDH1 and IDH2 based on PCR analysis [12]. These methods are very expensive and time-consuming; furthermore, extensive processing of the biological sample is needed.

To address the necessities of clinical practice for the molecular recognition and determination of IDH1 and IDH2 in biological samples, a reliable, fast, and cost-effective screening method is developed based on the utilization of stochastic microsensors—the only type of sensors able to perform qualitative and quantitative analysis [13][14][15]. The stochastic microsensors were based on immobilization of a solution of PIX in SWCNT and MWCNT decorated with Cu and Au nanoparticles. Carbon nanotubes (CNT) have the good conductivity (improved by the addition of Cu and Au nanoparticles) and good chemical stability needed to maintain in shape the channels of the stochastic

microsensors [16][17]. Protoporphyrin IX is well known for its ability to form the molecular aggregates and stable channels needed for stochastic sensing [18].

2. Morphological Characterization of the CNT Pastes

The morphology of the pastes (CuAuNP-PIX/SWCNT and CuAuNP-PIX/MWCNT) that contain the necessary channels for the stochastic response is shown in Figure 1 (a.2 and b.2). To evaluate the elemental composition, the quantification of the elements, and their distribution in the material, semi-quantitative analysis was performed by EDX. Moreover, from the mapping, the uniform distribution of the elements in both modified pastes may be seen in Figure 1 (a.1 and b.1).



(**a**) (**b**)

Figure 1. Elemental mapping (a.1, b.1), surface morphology (a.2, b.2), and EDX spectrum of the pastes based on: (**a**) CuAuNP-PIX/SWCNT and (**b**) CuAuNP-PIX/MWCNT.

3. Response Characteristics of the Stochastic Microsensors

The response characteristics of the stochastic microsensors used for molecular recognition of IDH1 and IDH2 are shown in Table 1. The signatures obtained for IDH1 and IDH2 were different for each of these microsensors, thus demonstrating the ability of the microsensors to perform the molecular recognition of IDH1 and IDH2 in the biological samples.

Table 1. The response characteristics of the stochastic microsensors used for the molecular recognition of IDH1 and IDH2.

Stochastic Microsensor Based On	Signature of IDH t_{off} (s)	Linear Concentration Range (ng mL^{-1})	Calibration Equations; The Correlation Coefficient, r *	Sensitivity (s µg mL^{-1})	LOQ (fg mL^{-1})
CuAuNP-PIX/SWCNT			IDH1		
	0.7	1×10^{-5}–1×10^{2}	$1/t_{on} = 0.03 + 1.48 \times C$; r = 0.9999	1.48	10
			IDH2		
	1.4	5×10^{-8}–5×10^{2}	$1/t_{on} = 0.03 + 7.30 \times 10^{4} \times C$; r = 0.9999	7.30×10^{4}	5×10^{-3}
CuAuNP-PIX/MWCNT			IDH1		
	1.5	1×10^{-5}–1×10^{2}	$1/t_{on} = 0.04 + 9.58 \times 10^{5} \times C$; r = 0.9989	9.58×10^{5}	10
			IDH2		
	0.7	5×10^{-8}–5×10^{2}	$1/t_{on} = 0.16 + 1.50 \times 10^{7} \times C$; r = 0.9999	1.50×10^{7}	5×10^{-3}

* <C-concentration > = µg mL^{-1}; $<t_{on}>$ =s; LOQ—limit of quantification.

Utilization of SWCNT or MWCNT did not influence the linear concentration ranges for the assay of IDH1 (1×10^{-5}–1×10^{2} ng mL^{-1}) and IDH2 (5×10^{-8}- 5×10^{2} ng mL^{-1}), as well as the limits of quantification for IDH1 (10 fg mL^{-1}) and IDH2 (5×10^{-3} fg mL^{-1}), but it influenced the sensitivity of the proposed stochastic microsensors: the highest sensitivity was obtained when MWCNT was used for the molecular recognition of IDH1 (9.58×10^{5} s µg mL^{-1}) and IDH2 (1.50×10^{7} s µg mL^{-1}). Accordingly, the stochastic microsensor of choice for the molecular recognition and quantification of IDH1 and IDH2 is the one based on CuAuNP-PIX/MWCNT.

Compared with the disposable stochastic sensors proposed before [11] (Table 2), a wider linear concentration range and a lower limit of quantification versus the disposable Chitosan/Cu nanolayer-based stochastic sensor was recorded for the assay of IDH1. Moreover, a lower limit of quantification was achieved for the assay of IDH2 with the stochastic sensors based on CNT. Analyses with sensors based on CNT are more cost-effective than those performed using the disposable stochastic sensors because the former can be kept and used continuously for more than one month.

Table 2. The comparison of stochastic microsensors for the assay of IDH1 and IDH2.

Stochastic Microsensors	Linear Concentration Range (ng mL^{-1})	Sensitivity (s µg mL^{-1})	LOQ (fg mL^{-1})	Reference
Disposable Chitosan/Cu nanolayer		IDH1		[11]

Stochastic Microsensors	Linear Concentration Range (ng mL^{-1})	Sensitivity (s µg mL^{-1})	LOQ (fg mL^{-1})	Reference
Disposable Chitosan/GR * nanolayer	1×10^{-4}–1×10^{2}	1.00×10^{7}	10^{2}	This work
		IDH2		
	5×10^{-7}–5×10^{2}	9.51×10^{5}	5×10^{-1}	
		IDH1		
	1×10^{-8}–1×10^{2}	3.77×10^{7}	10^{-2}	
		IDH2		
	5×10^{-8}–5×10^{2}	1.88×10^{7}	5×10^{-2}	
		IDH1		
Disposable Chitosan/GR-Cu composite nanolayer	1×10^{-5}–1×10^{2}	2.73×10^{7}	10^{-1}	
		IDH2		
	5×10^{-8}–5×10^{2}	4.44×10^{6}	5×10^{-2}	
		IDH1		
CuAuNP-PIX/SWCNT	1×10^{-5}–1×10^{2}	1.48	10	
		IDH2		
	5×10^{-8}–5×10^{2}	7.30×10^{4}	5×10^{-3}	
		IDH1		
CuAuNP-PIX/MWCNT	1×10^{-5}–1×10^{2}	9.58×10^{5}	10	
		IDH2		
	5×10^{-8}–5×10^{2}	1.50×10^{7}	5×10^{-3}	

* GR = graphene.

Ten of each type of microsensor were designed and used for 1 month for the assay of IDH1 and IDH2. In this period of time, the sensitivities for IDH1 and IDH2 were recorded. For each type of microsensor, the measurements performed during one day showed that the RSD% values for the variation of the sensitivities recorded for 10 microsensors were 0.10% for IDH1 and 0.15% for IDH2 despite the type of microsensor, proving a highly reliable (reproducible) design of the proposed stochastic microsensors. When used for 1 month, the sensitivity variations were 0.37% for the assay of IDH1 and 0.40% for the assay of IDH2 despite the type of microsensor, proving the stability of the microsensors in time.

The selectivity of the stochastic microsensors is given by the signatures (t_{off} values) recorded for different analytes. The signature of the analyte and the possible interference depends on several factors such as molecule size and conformation, deployment capacity, or speed of going in the channel; thus, the signature can act as an element of molecular recognition, contributing to the qualitative analysis of mixtures. The different signatures obtained for analytes such as IDH1, IDH2, heregulin-α, dopamine, epinephrine, and levodopa proved the selectivity of the proposed stochastic microsensor (Table 3).

Table 3. The selectivity of the stochastic microsensors.

Stochastic Microsensor Based On	t_{off} (s), Signature					
	IDH1	IDH2	Heregulin-α	Dopamine	Epinephrine	Levodopa
CuAuNP-PIX/SWCNT	0.7	1.4	0.2	1.9	3.0	2.5
CuAuNP-PIX/MWCNT	1.5	0.7	1.8	2.4	3.2	2.8

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