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# **Aligned copper nanowires as a cut-and-paste exclusive electrochemical transducer for free-enzyme highly selective quantification of intracellular hydrogen peroxide in cisplatin-treated cells**

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## **Introduction**

Metallic nanowires (MNWs) are important building blocks in nanotechnology. They are metallic or semiconducting 1-D nanostructures with cylindrical shape and one-dimensional anisotropic structure, small in diameter and large in surface to volume ratio (Rawtani et al., 2015). Electrocatalysis towards target molecules, large scale redox conversion and high stability and resistance to passivation are originated from the great surface area of the MNWs-based electrochemical detectors (Chan et al., 2015; García et al., 2014).

To date, the exploitation of these advantageous features has been mainly carried out by a drop casting approach. In it, a thin film of nanomaterial is randomly deposited on another conductive substrate, leading to a fragile distribution of nanomaterial on the electrode surface. Consequently, the analytical response is dependent on both the deposited film of nanomaterial and the conductive substrate, resulting in possible diffusion problems and poor analytical performance. Therefore, the development of novel nanomaterial-based arrangements supporting exclusively the transduction are highly needed.

On the other hand, the orientation of the MNWs is another important feature to exploit the high surface of these nanomaterials. To this end, the use of a magnet to align nickel nanowires has demonstrated a much better superior analytical performance in comparison with a random nanowire distribution (García and Escarpa, 2011). Polycarbonate membrane has also been used as a template on bare glassy carbon electrode (GCE) to electrodeposited metallic nanowires (Liu et al., 2013), attached to the bare electrode using pressure or including Nafion as a polyelectrolytic glue (Gambirasi et al., 2011; Stortini et al., 2014). However, all these approaches uses an extra conductive substrate, GCE as electrodic support, which has several disadvantages such as, GCE is not a miniaturized electrode, and requires tedious polish procedure between experiments, which are not suitable for fast clinical diagnosis. In addition, the modification methods become in fragile configurations due to the glue chemicals used and the analytical signal is still dependent on the nanomaterial and the electrode support.

As an alternative to those approaches, in this work, we present vertically aligned copper nanowires (v-CuNWs), as a cut-and-paste flexible electrochemical sensor without extra fastening, acting as exclusive transducers of the electrochemical signal. This novel approach is applied to reliably and free-enzyme quantify the intracellular hydrogen peroxide content in Cisplatin-treated human renal proximal tubular epithelial (HK-2) cells.

The role and reliable quantification of intracellular hydrogen peroxide in Cisplatin (cis-diamminedichloroplatinum (II)) nephrotoxicity during cancer therapy still constitutes an unexplored and fascinating application. Nowadays, only few reports have suggested the pathological role of hydrogen peroxide among the other intracellular reactive oxygen species (ROS) generated in the kidneys during treatment with Cisplatin (Baek et al., 2003; Lieberthal et al., 1996; Tsutsumishita et al., 1998) and its reliable intracellular quantification in Cisplatin-treated renal proximal tubular cells has not been still studied. In addition, quantification of intracellular  $H_2O_2$  is essential to determine the role of  $H_2O_2$  in the mechanism of drug cytotoxicity, drug therapeutic effect, ROS-related physiological or pathological effects at the cell level, etc (Rawson et al., 2015). Therefore, the development of fast and selective methods for monitoring these processes with capabilities to assess the effect of Cisplatin becomes of extreme importance.

Although, several electrochemical sensors have been proposed for hydrogen peroxide detection, involving carbon surfaces (Xu et al., 2011; Lin et al., 2009) and electrocatalysts such as copper (Sophia and Muralidharan, 2015), nickel (Ji et al., 2013), cobalt (Jia et al., 2009), gold (Wang et al., 2015) and silver (Lee et al., 2016); however, only a few of them are based exclusively on nanomaterials, and they are presenting complex and non oriented architectures. Other optical nonenzymatic sensors based on carbon nanomaterials, carbon nanotubes, carbon nanodots, possessing intrinsic peroxidase-like activity, have been also proposed but require larger concentrations of  $H_2O_2$  to be detected. (Song et al., 2010; Shi et al., 2011) To our best knowledge, the v-CuNWs as a cut-and-paste flexible electrochemical sensor without extra supporter, acting as exclusive transducers of the electrochemical signal, constitutes a pioneer approach not only for hydrogen peroxide monitoring in cisplatin-treated HK-2 cells but for future in vitro diagnosis. In addition, the relevance of  $H_2O_2$  among other ROS generated by Cisplatin, in nephrotoxicity, has not been clearly established; being our work the first one that analyzes the time course of the  $H_2O_2$  release of Cisplatin-treated human renal proximal tubular cells to the extracellular medium avoiding any cellular lysis procedure.

## Material and methods

### *Apparatus*

Autolab PGSTAT 12 potentiostat (Eco Chemie, Utrecht, The Netherlands) was used to carry out amperometric detection. Autolab  $\mu$ Autolab type II potentiostat (Eco Chemie, Utrecht, The Netherlands) to electrosynthesize the nanowires.

### *Materials, standards and cell samples*

Polycarbonate membranes were purchased from Whatman, Maidstone, UK (25 mm diameter and 0.3  $\mu$ m pore diameter).  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (7791-20-0 Sigma-Aldrich) solution of 1 M in 0.3 M  $\text{H}_3\text{BO}_3$  (45,6671 Fluka) was used to fabricate the v-CuNWs.  $\text{CH}_2\text{Cl}_2$ ,  $\text{C}_3\text{H}_8\text{O}$  and  $\text{C}_2\text{H}_6\text{O}$  (75-09-2, 67-63-0, 64-17-5 Sigma-Aldrich), were used in the membrane removing and washing steps, respectively. Hydrogen peroxide (30% w/v) and Horseradish peroxidase (HRP) type VI-A (Sigma-Aldrich) dissolved in 100 mM  $\text{NaH}_2\text{PO}_4$  pH 7.4 was used. Cisplatin and propidium iodide were purchased from Sigma (St. Louis, MO).

Annexin-V was purchased from BD Pharmingen (San Diego, CA, USA) and Cell Tracker<sup>TM</sup> CM-Dil was from Invitrogen (Carlsbad, CA, USA).

Human renal proximal tubular epithelial (HK-2) cells, were purchased from American Type Culture Collection (ATCC) (Rockville, MD).

### *Fabrication of v-CuNWs*

v-CuNWs electrodes were fabricated by template electrodeposition protocol using a cyclopore polycarbonate membrane (0.3  $\mu$ m pore diameter) (Garcia et al., 2015; Martin, 1996). To this aim, one side of the membrane was sputtered with a 75 nm gold film, which was used as the working electrode during the electrodeposition. After that, the golden side of the membrane was placed in the electrochemical cell contacting with an aluminium foil (to create electric contact). A Pt wire and an Ag/AgCl (3 M KCl) were used as counter and reference electrodes. Copper nanowires were electrodeposited at  $-1.0$  V for  $-30$  C on the pores of the membrane (Garcia et al., 2015). The membrane was next, cut with the desired shape (circle of 4 mm diameter) with a biopsy punch (Biopunch, Redding, US) and stuck in double-sided adhesive tape (Fixo, Spain). After that, the membrane was removed to obtain the free v-CuNWs by immersing in dichloromethane 30 min and then, subsequently, gently rinsed in isopropanol, ethanol and water.

The adhesive tape, with orientated nanowires was directly stuck as working electrode and then pasted into a ceramic screen-printed platform (DS 110, Dropsens, Oviedo, Spain), in which the carbonworking electrode was completely removed and replace with the vCuNWs. Conductive silver paint (Electrolube, UK) to create the electric contact and epoxy protective overcoat (242-SB, ESL Europe) for the isolation, were used.

### *Characterization of v-CuNWs*

In order to characterize v-CuNWs electrodes, they were prior sputtered with gold, before micrographs were taken by a scanning electronic microscopy DSM 950. Photoelectron spectra were recorded in a VG ESCALAB 200R electron spectrometer provided with hemispherical electron analyzer and non-monochromated MgK $\alpha$  (h.v.=1253.6 eV) X-ray source powered at 120 W. The kinetic energy scale was calibrated using Au4f<sup>7/2</sup> at 84.0 eV and Cu2p<sup>3/2</sup> at 932.7 eV from foil samples. Overview spectrum (200–1300 eV) and high resolution C1s, O1s (20 eV) and Cu2p (50 eV) energy regions were scanned a number of times to get good signal-to-noise ratios. Peak fitting was performed by means of XPS peak software. After subtraction of a Shirley background, the peaks were fitted using a non-linear, least squares routine with mixed Gaussian-Lorentzian functions. No presputtering was carried out prior to acquiring the spectra and base pressure within the analysis chamber was maintained below  $4 \times 10^{-9}$  mbar during data acquisition.

### *Electrochemical sensing and biosensing for H<sub>2</sub>O<sub>2</sub>*

The electrochemical sensing of H<sub>2</sub>O<sub>2</sub> generated from cell culture treated with Cisplatin was monitored by chronoamperometry at  $-0.20$  V on v-CuNWs using 50  $\mu$ L of cell culture.

To evaluate the v-CuNWs selectivity towards H<sub>2</sub>O<sub>2</sub> and performance of the sensor, another methodology based on a biosensor was studied. The electrochemical bio-sensing of H<sub>2</sub>O<sub>2</sub> generated from the enzymatic reaction was directly performed in situ on the carbon-screen printed electrodes (DS 110, Dropsens, Oviedo, Spain) into a 50  $\mu$ L drop (43  $\mu$ L of the sample or H<sub>2</sub>O<sub>2</sub>; 2  $\mu$ L of a 4 mg/mL HRP and 5  $\mu$ L of 10 mM hydroquinone, all in 100 mM NaH<sub>2</sub>PO<sub>4</sub> pH 7.0). In this approach, the enzyme is not covalently immobilized onto the electrodic surface of the electrode.

#### **2.6. Cell treatment and morphology**

Cells treated with Cisplatin were provided by the research group of Prof. Javier de Lucio at the Faculty of Medicine (University of Alcala, Spain).

Studies on the toxic effect of Cisplatin were performed on HK-2 cells cultured in Dulbecco's Modified Eagle Medium (DMEM F-12) with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin/ amphoterycin B and 1% glutamine (Invitrogen, Carlsbad, CA) and 1% Insulin-Transferrine-Selenium (Sigma, St. Louis, MO). 24 h before cisplatin treatment (25  $\mu$ M), cells were trypsinized and plated at 70– 80% confluency.

Cell morphology in Cisplatin-treated cells was studied directly by confocal laser scan microscopy after staining with Cell Tracker<sup>TM</sup> CMDil and DAPI. Cisplatin-induced apoptosis was quantified by flow cytometry with a FACSCalibur cytometer (Becton Dickinson, USA) in trypsinized cells which were incubated with annexin V/fluorescein isothiocyanate and propidium iodide solution. The methodological details of these studies have been described recently elsewhere (Fernández-Martínez et al., 2016).

Each experiment was repeated at least three times. The results are expressed as the mean  $\pm$  SD. They were subjected to one way analysis of variance (ANOVA) following by the Bonferroni's test for multiple comparisons. The level of significance was set at  $P < 0.05$ .

## **Results and discussion**

### *Sensor design and development*

Fig. 1A shows a scheme for the design and development of vCuNWs film as a cut-and-paste exclusive electrochemical transducer. Membrane containing electro synthesized CuNWs is cut and pasted on a cello-tape strip, and then in situ dissolved obtaining a flexible CuNWs forest, acting as exclusive transducer without any extra support.

Fig. 1B (top) shows a picture of the CuNWs pasted into flexible adhesive tape. It is worth to mention that the circle design could be changed to any tailored shape and pasted on any other surface. After in situ membrane removal, a forest of vertically oriented v-CuNWs with an average diameter of 300 nm was clearly observed (see Fig. 1B bottom) without application of external magnetic fields, extra conductive supports or chemical additives. Energy dispersive spectroscopy (EDS) analysis confirmed the homogeneity of the v-CuNWs, revealing that they were 100% Cu, thereby confirming the convenience and simplicity of the electrochemical fabrication.

Therefore, this approach allows simple and versatile design of vertically-oriented copper nanowires.

The elemental composition of the electrode surface and the chemical environment of the atoms were evaluated by X-ray photoelectron spectroscopy (XPS) (See Fig. S1).

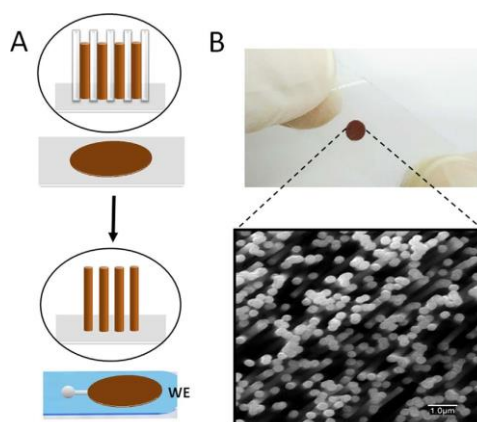


Fig. 1. (A) Schematic of the fabrication procedure of flexible aligned v-CuNWs (from top to bottom): v-CuNWs (electrosynthesized in polycarbonate membrane) are cut (with a tailored shape), pasted on a strip of cello-tape and in situ removed from the membrane on the non-conductive substrate. (B) Photograph of a circular pasted v-CuNWs on adhesive tape (top). SEM image of v-CuNWs electrode (Scale bar 1  $\mu\text{m}$ ) (bottom).

Table 1

Binding energies (eV) of core-levels non-oxidized and oxidized copper nanowires.

Sample	C1s	Cu2p <sup>3/2</sup> (%)	O1s
v-CuNWs	284.8	932.6 (30) 934.5 (70)	532.4
v-CuNWs oxidized	284.8	932.7 (18) 934.6 (82)	532.5

Note: Parentheses mean percentage of each component (metallic copper and oxidize copper species). Bold means the component related to oxidize copper species.

As the XPS survey scan allows identify unequivocally the elements present on solid surfaces, low resolution, wide energy scans were recorded for all the samples. In addition, high-resolution C1s, O1s and Cu2p core-level spectra were recorded and the corresponding binding energies are summarized in Table 1.

The Cu2p core-level spectra of the electrode samples are shown in Fig. S1A-B. The Cu2p emission was satisfactorily curve-resolved with three sets of doublets separated by about 17.5 eV: (i) one intense doublet, which major component (Cu2p<sup>3/2</sup>) appears at 934.5–934.6 eV is due to oxidized copper species, (ii) a minor one which Cu2p<sup>3/2</sup> component is observed at 932.6–932.7 eV due to metallic copper; and (iii) a third doublet high binding energy side of the principal 2p components which is the corresponding satellite lines of each Cu2p components. As Cu<sup>0</sup> and Cu<sup>+</sup> do not display satellite line, the observation of such satellites was unambiguous probe of the presence of a certain proportion of CuO species in the sample. By looking at the intensities, the proportion of CuO is higher

in the oxidized v-CuNWs than in the non-oxidized v-CuNWs. On the contrary, the fraction of metallic copper is slightly higher in the non-oxidized v-CuNWs than in the oxidized v-CuNWs. For the easy identification, the doublet belonging to metallic copper is plotted in light blue colour.

Then, v-CuNWs were carefully evaluated towards the  $\text{H}_2\text{O}_2$  detection. Fig. 2 shows the hydrodynamic voltammograms obtained on vCuNWs as well as with other commercial electrodes (Prussian blue and thick-film Cu). In the inset is shown the chronoamperometry signals at the selected potential  $-0.20$  V where the analytical current is measured at 200 s. Interestingly, v-CuNWs electrode provided the better electrochemical performance for  $\text{H}_2\text{O}_2$  detection, even in comparison with thick-film Cu electrode offering a very good sensitivity at low potentials ( $-0.20$  V) without use any mediator as Prussian Blue. In both copper electrodes, the Cu oxidation to Cu oxides (previously optimized  $+0.70$  V during 150 s in 0.1 M NaOH) was also essential to obtain the analytical signal. As expected, due to the electrocatalysis mechanism, in absence of these copper oxides, non-oxidized v-CuNWs did not give analytical signal. XPS studies confirm the presence of copper oxides in those v-CuNWs upon controlled oxidation.

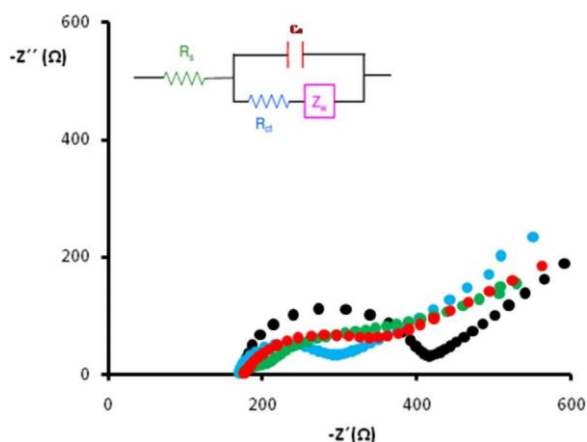


Fig. 2. Hydrodynamic voltammograms of 1 mM  $\text{H}_2\text{O}_2$  in v-CuNWs (red), commercial thick-film Cu (green) and Prussian blue (blue) electrodes. Inset shows the chronoamperograms at the selected potential ( $-0.20$  V), where the analytical current is measured at 200 s.

Fig. S2 shows the calibration performance of v-CuNWs at  $-0.20$  V. The sensor exhibited an excellent linearity ( $r=0.990$ ) in the concentration range examined ( $0.5$ – $800$   $\mu\text{M}$ ), with a good analytical sensitivity ( $7.4$   $\mu\text{A}/\text{mM}$ ) and limit of detection (LOD) ( $0.4$   $\mu\text{M}$ ,  $S/N=3$  criteria). LOD was calculated as  $3 s/\text{slope}$ , where  $s$  is the

standard deviation obtained during the measurement of the lowest concentration (0.5  $\mu$ M; n=10).

Intra-electrode repeatability was also evaluated for two  $\text{H}_2\text{O}_2$  concentration levels (5  $\mu$ M and 1 mM), yielding values of relative standard deviations (RSDs) < 7% (n=5). The inter-electrode reproducibility was also lower of 8% (n=5), indicating an excellent reproducibility of the fabricated electrodes.

#### *Application of v-CuNWs for intracellular hydrogen peroxide quantification in cisplatin- treated cells*

The effect of exposure time of Cisplatin was evaluated towards the  $\text{H}_2\text{O}_2$  quantification in HK-2 cells treated with Cisplatin using vCuNWs.

First, cell morphology is shown in Fig. 3 in both, control (3 A), and at 24 h (3B) using confocal laser scan microscopy. Cell death with apoptotic-like nuclear morphology was only observed in cells treated with cisplatin for 24 h (Fig. 3B), while it was not for the initial 8 h (Fig. 3A), which indicates that cell death starts after 8 h from the treatment with cisplatin. Furthermore, Cisplatin-induced apoptosis was quantified by flow cytometry in dose-response and time-response experiments in cells incubated with annexin V/propidium iodide (Fig. 3C). It is worth to mention that the toxic effects, including those caused by  $\text{H}_2\text{O}_2$ , happen immediately and leads to the death of the cell. However, the increase in intracellular  $\text{H}_2\text{O}_2$  (and in other toxic compounds) induced by cisplatin does not lead immediately to the death of the cell.

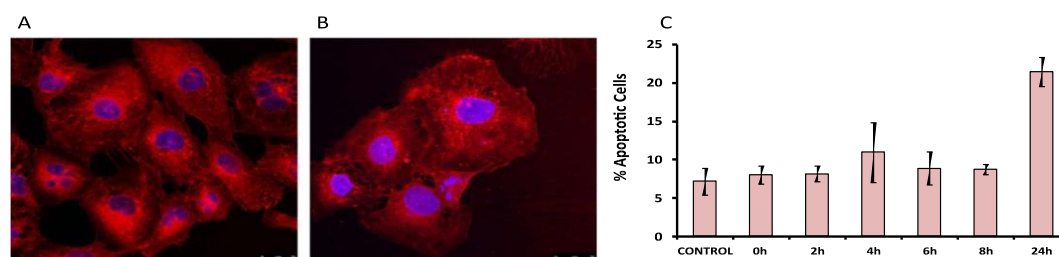


Fig. 3. Effects of Cisplatin on human proximal tubular HK-2 cells treated during (A) initial stages, from 0 to 8 h and (B) after 24 h of treatment. Confocal photomicrographs showing Cisplatin-induced apoptotic-like nuclear morphology (at original magnification, 10 $\times$ ) after staining with Tracker<sup>TM</sup> CM-Dil (red) and DAPI (blue). (C) Staining-based apoptosis determination. The bars show the sum of the percentage of annexin V+/propidium iodide+ cells (stained cells) and the percent of annexin V+/propidium iodide+ cells (stain + cells) at different times. Cells were treated with 25  $\mu$ M Cisplatin for up to 24 h.

Fig. 4 shows the extracellular  $\text{H}_2\text{O}_2$  levels (blue bars) obtained for mHK-2 cells after cis-platin treatment at different time intervals (0, 2, 4, 6, 8 and 24 h) using v-CuNWs as a free-enzyme approach. Extracellular  $\text{H}_2\text{O}_2$  levels increased up to 6 h, keeping constant until the first 24 h. To demonstrate that this extracellular  $\text{H}_2\text{O}_2$  was intracellular generated and, consequently, it diffused from the cells to extracellular media, control experiments were also carried out. To this end, we measured the intracellular  $\text{H}_2\text{O}_2$  from cells which were lysed and diluted in a volume equal to that of extracellular medium (Fig. 4, red bars). Identical levels for intracellular  $\text{H}_2\text{O}_2$  were obtained in comparison with those obtained for extracellular  $\text{H}_2\text{O}_2$  using v-CuNWs. The  $\text{H}_2\text{O}_2$  levels showed excellent correlation values ( $r=0.998$ ) at significant level ( $p=0.05$ ) including zero for intercept ( $-0.001 \pm 0.005$ ) and 1 for slope ( $0.99 \pm 0.07$ ) indicating an identical extra and intra cellular levels. Consequently, we are estimating the intracellular hydrogen peroxide towards simple extracellular assessment.

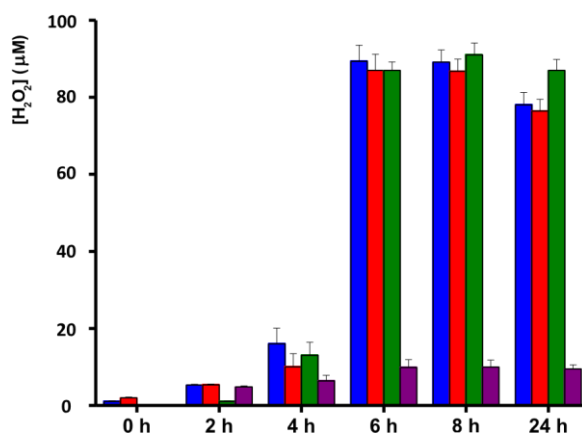


Fig. 4. Levels for extracellular (blue bars), intracellular (red bars)  $\text{H}_2\text{O}_2$  using v-CuNWs sensor and extracellular  $\text{H}_2\text{O}_2$  (green bars) using electrochemical biosensor in cultured HK-2 cells treated with Cisplatin at different time intervals (0, 2, 4, 6, 8 and 24 h). Levels for extracellular  $\text{H}_2\text{O}_2$  (purple bars) using v-CuNWs in cultured HK-2 cells treated with Cisplatin and HRP at different time intervals (0, 2, 4, 6, 8 and 24 h). Results are given as mean values  $\pm$  standard deviation ( $n=3$ ).

Furthermore, to evaluate the v-CuNWs selectivity towards  $\text{H}_2\text{O}_2$  and performance of the sensor, cell cultures treated with Cisplatin were also analyzed using an established biosensor approach, using HRP, which is highly selective for  $\text{H}_2\text{O}_2$  (Xiao et al., 1999) (see Fig. 4, green bars), enzyme-based approach. Both approaches our proposed freeenzyme v-CuNWs and the HRP enzyme-based one provided identical  $\text{H}_2\text{O}_2$  levels. Indeed, the correlation method provided excellent results ( $r=0.998$ ) at significant level ( $p=0.05$ ) including zero for intercept ( $-0.003 \pm 0.007$ ) and 1 for slope ( $1.1 \pm 0.1$ ) indicating not only a very good selectivity and performance but also, a good accuracy of the method (since 0 and

1 belongs to intercept and slope confidence intervals, respectively) of the proposed v-CuNWs-based method.

In addition, another control at real time was also explored. In this control, during the cell incubation with cisplatin, a HRP enzyme was added to consume the H<sub>2</sub>O<sub>2</sub> produced at real time during the death process. Interestingly, for all the incubation times a large decrease of the hydrogen peroxide was obtained confirming the in situ H<sub>2</sub>O<sub>2</sub> generation during the treatment (see Fig. 4, purple bars).

The untreated and treated cell cultures with Cisplatin (4 and 8 h) were also spiked with 500  $\mu$ M H<sub>2</sub>O<sub>2</sub>. Table 2 lists the quantitative recoveries obtained revealing a very good accuracy during the H<sub>2</sub>O<sub>2</sub> determination in the cell samples. It is worth to mention that, the control using untreated cells cultures did not give any detected signal, see Table 2. To further confirm the reproducibility of the method, three independent cell culture experiments were performed showing an excellent reproducibility (RSDs < 8%, n=3).

Table 2

Determination of hydrogen peroxide in HK-2 tumor cell cultures treated with cisplatin using v-CuNWs.

Sample	H2O2 added ( $\mu$ M)	H2O2 sample ( $\mu$ M)	H2O2 Total ( $\mu$ M)	Recovery (%)
Control	500	0	500	100.2 $\pm$ 0.5
4 h		16	516	101 $\pm$ 1
8 h		89	589	100 $\pm$ 1

Results are given as mean values  $\pm$  standard deviation (n=3).

Consequently, previous results demonstrate that H<sub>2</sub>O<sub>2</sub> is released from the HK-2 cells after they are treated with cis-platin and is accumulated in the extracellular medium. This is a valuable finding since v-CuNWs are able to evaluate the released intracellular H<sub>2</sub>O<sub>2</sub> in a very simple way avoiding any cellular lysis procedure, thereby contributing to assess the role of H<sub>2</sub>O<sub>2</sub> in cell death. Also, worth to mentioning, we studied the stability of exogenously added H<sub>2</sub>O<sub>2</sub> in culture medium maintained at 37 °C and darkness, in the same conditions in which we did the experiments with cells, finding that, over a period of 24 h, H<sub>2</sub>O<sub>2</sub> concentration remained constant (control results not shown). This indicated its chemical stability along all the process and consequently the reliability of the v-CuNWs to detect this molecule. Therefore, these results suggest the potential of the v-CuNWs as a highly selective and accurate free-enzyme sensor for

monitoring intra cellular hydrogen peroxide. In addition, the  $\text{H}_2\text{O}_2$  levels found in our work are in agreement with previous reported in relevant literature (Aihara et al., 2003; Han et al., 2005; Wang and Sanders, 2007).

On the other hand, although  $\text{H}_2\text{O}_2$  scavengers have been shown to prevent Cisplatin-induced renal proximal tubular cell death, (Baek et al., 2003; Pabla and Dong, 2008; Lieberthal et al., 1996) the relevance of  $\text{H}_2\text{O}_2$  among other ROS generated by Cisplatin, in nephrotoxicity, has not been clearly established. In fact, excepting for one study (Salahudeen et al., 1998), there are no reports on the generation of  $\text{H}_2\text{O}_2$  upon treatment of renal proximal tubular cells with Cisplatin. In this way, to the best of our knowledge, our work is the first one to analyze the time-course of the release of  $\text{H}_2\text{O}_2$  to the extracellular medium in Cisplatin-treated human renal proximal tubular cells avoiding any cellular lysis procedure, which constitutes valuable information.

## **Conclusion**

We have demonstrated that aligned v-CuNWs pasted in cello tape, supports exclusively the electrochemical transduction, they are easily adaptable to any detection configuration, and their fabrication can be afforded in any laboratory, without need of cleanroom facilities. In addition, this free-enzyme approach is compatible with mass-production, disposability and can be straightforward tailored by electrodepositing different metals, polymers or even combinations with biological material.

v-CuNWs has demonstrated to be a simple, versatile and highly selective analytical tool for accurate quantification of intracellular hydrogen peroxide in cell cultures without a lysis procedure which simplifies notably the procedure and enhances the sensor performance using extremely low cell culture volumes and simple instrumentation without need of qualified users. Furthermore, the high surface area and the high selectivity of v-CuNWs could be suitable for measurement of  $\text{H}_2\text{O}_2$  in more complex media environments such as blood.

In addition, this approach highly improves the cell death monitoring in comparison with the well-established flow cytometry: 6 h vs. 24 h. Therefore, this approach opens novel avenues for monitoring cell death process, the damage on kidney cells, and its implication in cancer therapy, such as the future reduction in the Cisplatin levels.

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