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RESEARCH ARTICLE

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Development of an Electrochemical Metal-Ion Biosensor Using Self-Assembled Peptide Nanofibrils

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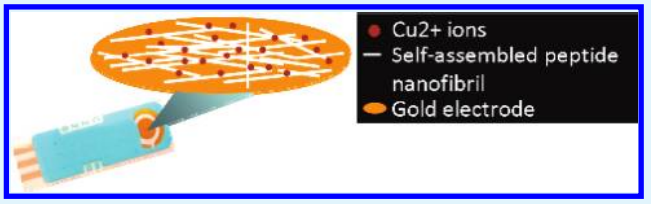
Supporting Information

ABSTRACT: This article describes the combination of self-assembled peptide nanofibrils with metal electrodes for the development of an electrochemical metal-ion biosensor. The biological nanofibrils were immobilized on gold electrodes and used as biorecognition elements for the complexation with copper ions. These nanofibrils were obtained under aqueous conditions, at room temperature and outside the clean room. The functionalized gold electrode was evaluated by cyclic voltammetry, impedance spectroscopy, energy dispersive X-ray and atomic force microscopy. The obtained results displayed a layer of nanofibrils able to complex with copper ions in solution. The response of the obtained biosensor was linear up to 50 μM copper and presented a sensitivity of 0.68 μA cm^{-2} μM^{-1}. Moreover, the fabricated sensor could be regenerated to a copper-free state allowing its reutilization.



KEYWORDS: self-assembly, peptide, nanofibrils, electrochemical biosensor, copper, biomaterial

INTRODUCTION

The self-organization of biological molecules into well-defined structures associated with functionality is a constant source of inspiration for scientists.[1,2] Moreover, the convergence of self-assembled materials with analytical techniques potentially renders possible a range of new useful applications.[3−6] Substances such as DNA, RNA, proteins and peptides as well as the structures and forms that these molecules take are the basis for the development of novel materials with numerous possibilities with regard to applications in the field of bionanotechnology, such as biosensors, bioelectronics or drug delivery systems.[7−10]

Self-assembled peptides are biocompatible building blocks displaying chemical diversity, flexibility and stability.[11,12] These types of peptides are able to organize into nanostructures such as fibers, tubes or particles under mild conditions in a low-cost process.[5,13,14] This study focuses on amyloid-type nanofibrils based on an octapeptide building block derived from a natural fibrous protein, that is, the adenovirus fiber, which was used to modify the surface of gold electrodes. These short synthetic peptides have been found to self-assemble into amyloid-type fibrils in solution,[15] and the modified electrodes were used for the detection of copper ions in solution.

Peptide biomaterials can be very effective and specific ligands for a variety of metal ions.[16] They contain donor atoms present at the peptide backbone and at the amino acid side chain, which are able to complexate to metal ions.[17] This feature and the possibility of tuning the amino acid sequence that forms the nanofibers opens several possibilities of employing the octapeptides as biorecognition elements for the detection of metal ions. Metals such as copper or zinc are essential for life but can be toxic in high doses due to their interfering with the normal function of metalloenzymes and key biochemical pathways. This results in symptoms such as nausea and vomiting, or in the presence of diseases such as the Wilson disease.[18,19] Therefore, the ability to monitor concentrations of metal ions becomes important in providing a means to assess toxicity levels in samples like drinking water where the maximum amount of copper allowed is below 30 μM.[20]

Metals such as copper have previously been detected through atomic absorption spectroscopy, coupled plasma-mass spectrometry and colorimetric detection.[21−23] These methods exhibit good sensitivity, selectivity and accuracy and are able to report reliable multimeasurements. However, drawbacks include their use being expensive due to the necessary equipment and trained personnel. Additionally, the techniques are laboratory-based, which limits on-site metal monitoring.

On the other hand, electrochemical biosensors for the detection of metals are very simple and require less complex instrumentation.[24,25] Sensors base on the modification of electrodes with oligopeptides and ion selective electrodes (ISE) were used for the electrochemical detection of copper resulting in biosensors with detection limits in the nanomolar range.[17,20,26−29] The combination of self-assembled peptide nanofibrils immobilized

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on gold electrodes and electrochemical techniques offer a good alternative for the detection of metal ions in solutions, mainly because of the simplicity of the system. However, studies using these type of biological one-dimensional (1D) nanostructures for the detection of metals are scarce.[9]

Herein, we report on the integration of electrochemical transducers with cysteine-containing self-assembled peptide nanofibrils for the fabrication of a copper ion biosensor. Through binding of the biological nanofibrils on the metal transducers, it was possible to obtain a strong affinity suitable for the development of an electrochemical biosensor. The fabricated biosensor displayed a linear range up to 50 μM with a sensitivity of 0.68 μA cm^{-2} μM^{-1}. Moreover, the sensor could be reused through regeneration of the immobilized nanofibril layers.

MATERIALS AND METHODS

Peptides and Chemicals. The peptides (free N-termini, amidated C-termini), with a degree of purity higher than 95%, were supplied by Eurogentec (Belgium). Solutions were prepared using double-distilled water obtained from a Milli-Q system (Millipore, Bedford, MA). $K_3Fe(CN)_6$ was purchased from Merck (Germany), and ammonium acetate was obtained from Fluka (Switzerland). $CuSO_4$, NaOH, $HClO_4$, KCl, NaCl, and $Ru(NH_3)_6Cl$ were purchased from Sigma-Aldrich (Germany).

All voltammetric and amperometric experiments were performed using an Autolab PGSTAT 12 (Metrohm, Netherlands) potentiostat interfaced with a computer controlled by General Purpose Electrochemical System, GPES v4.7 (Eco Chemie B.V.) as the interface. For the impedance studies, a Reference 600 potentiostat/galvanostat/ZRA (Gamry Instruments, Warminster, PA, U.S.A.) was used. All potential values were reported versus the reference electrode. The screen-printed electrodes (Au, Pt, C) were purchased from Dropsens (Asturias, Spain). Energy dispersive X-ray (EDX) analyses were performed with an Oxford Inca EDX system incorporated in a scanning electron microscope (SEM) from Oxford Instruments, U.K.

Preparation of Self-Assembled Peptide Nanofibrils. Lyophilized peptide powders were dissolved in double-distilled water and incubated at 4 °C. Peptide stock solutions were incubated during 6 days to allow the fibrils to form.

Modification of the Metal Electrodes. A 5 μL drop of a solution containing self-assembled nanofibrils was deposited on top of the metal working electrode and incubated overnight at 4 °C. The electrodes were washed with double-distilled water prior to the electrochemical measurements to remove any unbound nanofibrils.

Electrochemical Characterization and Regeneration of the Modified Electrodes. The nanofibril-modified electrodes were evaluated in a typical three-electrode glass cell. Three electrochemical techniques, i.e., cyclic voltammetry (CV), square wave voltammetry (SWV) and impedance spectroscopy, were used to characterize the modified electrodes. All electrochemical measurements were performed at room temperature.

For the CV experiments, the electrodes were immersed in a solution of 1.0×10^{-2} M $K_3Fe(CN)_6$ containing 0.1 M KCl and in a solution of 1.0×10^{-2} M $Ru(NH_3)_6Cl$ containing 0.1 M KCl. All CV measurements were performed at 100 mV/s. The impedance measurements were performed at the equilibrium potential of the couple, with 5 mV (rms) sinusoidal excitation amplitude. Measurements were carried out at 10 steps per decade in the appropriate frequency range. For the copper measurements, copper ions were accumulated on the NC-modified gold electrodes at open circuit potential by immersing the electrode into 5 mL of an aqueous solution of copper(II) sulfate in 50 mM ammonium acetate (pH 6.8) and 50 mM NaCl for electrochemical measurements by SWV. The pulse amplitude was 25 mV with a step of 4 mV and a frequency of 25 Hz. The SW voltamogramms were recorded between −250 and 500 mV. After the measurements, bound copper was eliminated from the NC-modified electrode at 500 mV during 20 s in a 0.1 M $HClO_4$

Table 1. Sequence of the Four Octapeptides Used for the Nanofibrils Synthesis

peptide name	sequence
NS	N-S-G-A-I-T-I-G
NC	N-C-G-A-I-T-I-G
CN	C-N-G-A-I-T-I-G
CS	C-S-G-A-I-T-I-G

RESULTS AND DISCUSSION

Preparation of Self-Assembled Nanofibrils. For the formation of the fibers, the lyophilized peptides were dissolved in double distilled water at a final concentration of 6 mg/mL and incubated at 4 °C for 6 days. Four octapeptides were used in this initial part of the study, and their sequences are presented in Table 1. As can be seen, the NC, CN and CS octapeptides were cysteine containing variants of the NS octapeptide. The NC, CN, and CS octapeptides were synthesized with cysteine substitutes only at the position of the asparagine (N) and serine (S) residues, which did not affect their capacity to self-assemble into nanofibrils.[30] Moreover, molecular dynamics simulations indicated that the cysteine residues remain exposed at the surface of the fibrils and available for interaction with metals.[31] Indeed, the cysteine variants were found to efficiently nucleate the formation of silver, gold, and platinum nanoparticles in solution.[30] After 6 days of incubation, the prepared solutions were used to modify the metal electrodes.

Electrode Modification. Screen-printed gold, platinum, and carbon electrodes were modified by depositing a 5 μL droplet of the self-assembled NC-nanofibril peptide solution. The modified electrodes were incubated overnight at 4 °C. After the incubation time, the modified electrodes were evaluated by cyclic voltammetry (CV) to test the efficiency of the modification. Prior to the CV measurements, the modified electrodes were rinsed with double-distilled water to remove any unbound nanofibrils. Subsequently, atomic force microscopy (AFM) was used to characterize the nanofibril-modified electrodes.

Figure 1 shows a topographical AFM image of an NC-modified screen-printed gold electrode, A, corresponding error signal, B and the line profile of figure 1A, C. On the NC-modified electrode, long lines with nanometer widths-corresponding to the immobilized nanofibrils-were observed all over the electrode surface. The line profile displayed on figure 1C suggests that the fibers have a diameter of approximately 4 nm. This value is in agreement with a previous report for the same type of nanofibers.[30]

Supporting Information Figure S1 displays the cyclic voltammograms of the bare electrodes, solid line, and the nanofibril-modified electrodes, dotted line, in a solution of 1.0×10^{-2} M $K_3Fe(CN)_6$ + 0.1 M KCl. Compared to their unmodified counterparts, the NC-modified electrodes presented a lower current indicating the formation of nanofibril layers bonded to the metal electrode surface. These layers rendered it difficult for the redox couple to reach the metal surfaces. When NC-modified gold, platinum and carbon electrodes were compared, it was clear

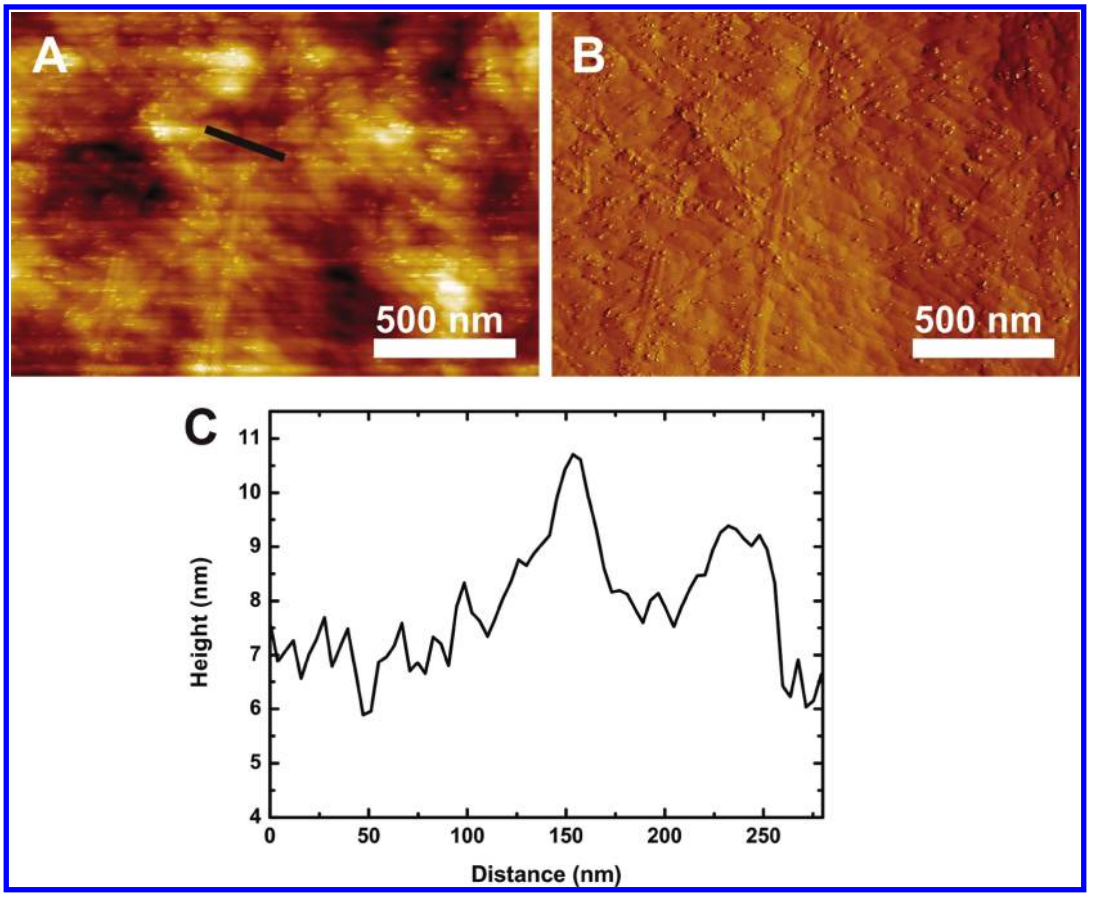

Figure 1. Dynamic mode AFM images showing (A) topography image of a NC-modified screen-printed gold electrode; (B) corresponding error signal of the image 1A; and (C) black line profile from 1A.

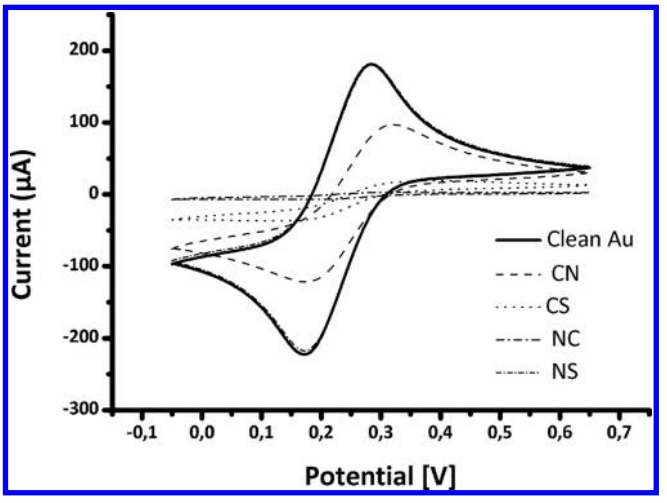

Figure 2. Cyclic voltammograms of screen-printed gold electrodes before, solid line, and after nanofibrils-modification, dotted lines, in a solution of 1.0×10^{-2} M $K_3Fe(CN)_6$ + 0.1 M KCl. Scan rate: 100 mV s^{-1}, applied potential vs Ag/AgCl.

that the NC nanofibrils were better attached to the gold electrode, since the obtained current value was lower than the ones for the modified Pt and C electrodes. This was expected due to the strong specific interactions between gold and the sulfur present in the cysteine groups of the NC fiber.[32,33] Based on this result, screen-printed gold electrodes were used for the rest of the study.

To evaluate the nanofibrils formed by each of the octapeptides presented in Table 1, cyclic voltammograms of Au electrodes modified which each of the fiber types were obtained, and the results are presented in Figure 2.

It was observed that the interaction between the nanofibrils containing a cysteine group was stronger than for the NS octapeptide that did not have cysteine in its sequence. Again, these results can be explained by the strong anchorage between the sulfur atoms present in cysteine and the gold surface. In fact, the NS-modified electrode did not display any insulation at all and its cyclic voltammogram was an almost exact match of the one obtained for the bare gold electrode.

In the case of the other three nanofibrils formed by the NC, CN and CS octapeptides, the degree of insulation was different. According to Figure 2, the degree of insulation given by the interaction of these nanofibrils with the gold electrode decreased in the order NC > CS > CN. A possible explanation for this behavior could be the position of the cysteine amino acid in the octapeptide sequence. Molecular dynamics simulations[31] suggested that the two N-terminal amino acids (aspargine and serine) of the basic building block NSGAITIG are not engaged in the core of the amyloid fibril, and therefore should be exposed and available for interaction. This was further confirmed experimentally, since peptides having cysteine substituted at these positions (the same ones used in this work) can self-assemble into fibrils that template metal nanoparticle

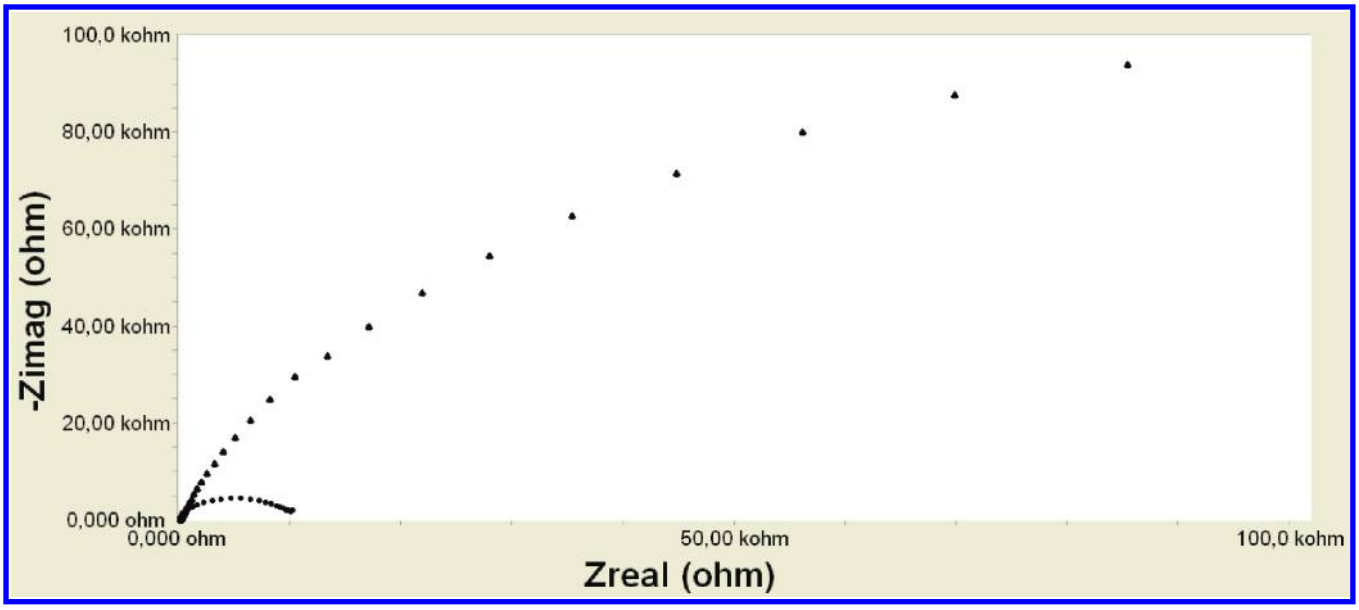

Figure 3. Impedance plots for a bare screen-printed gold electrode, solid circles, and its NC-modified counterpart, empty circles, in a solution of 1.0×10^{-2} M $K_3Fe(CN)_6$ + 0.1 M KCl. Frequency range: 0.1−10,000 Hz with a 5 mV rms signal.

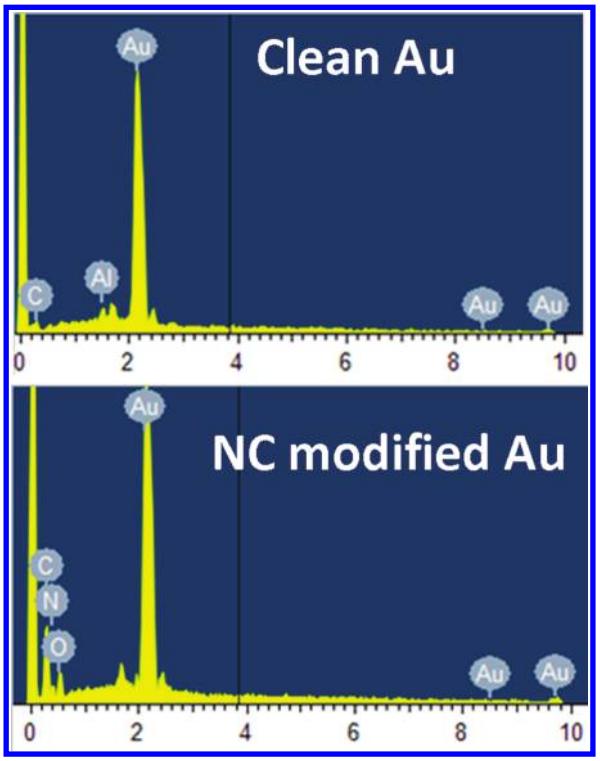

Figure 4. EDX spectra of a clean screen-printed gold electrode and its NC-modified counterpart.

formation in solution.[30] These fibrils can also attach to surfaces functionalized with free-thiol modifying reagents, such as iodoacetamide.[34,35] We therefore assume that the cysteine-containing fibrils attach to the gold surface through sulfur−gold interactions; however they still carry at their solution-exposed side cysteine groups available for interaction with copper ions. Based on these results, NC nanofibrils and gold electrodes were selected for the further development of the biosensor.

CV was further conducted with electroactive species, i.e., $K_3Fe(CN)_6$ and $Ru(NH_3)_6Cl$, to investigate the change in electrode behavior after modification of the Au electrode with NC-nanofibrils. As shown in Supporting Information Figure S2, the redox peaks decreased significantly when the NC nanofibrils were deposited on the bare Au electrode. This situation was similar for evaluation in both $K_3Fe(CN)_6$ and $Ru(NH_3)_6Cl$, which indicated a good insulation and the avoidance of positive or negative electrospecies passing to the electrode surface.

The following two pairs of electroactive species, that is, $Fe(CN)_6^{3-}$/ $Fe(CN)_6^{4-}$ and $Ru(NH_3)_6^{3+}$/ $Ru(NH_3)_6^{2+}$, were chosen to determine whether the decrease in current observed after the deposition of the NC nanofibrils was in fact due to the coverage of the electrode surface by the nanostructures and not the result of the rejection of the electroactive species by charges of the same sign that might be present on the nanofibril surface.[36]

Additional validation of the presence of NC-nanofibrils immobilized on the surface of the gold electrodes was achieved by impedance spectroscopy and energy dispersive X-ray (EDX). Figure 3 displays the complex impedance plots of the bare gold electrode and their NC-modified counterparts.

It was clear that the semicircle at high frequency, corresponding to the NC-modified Au electrode, was larger than that of the bare Au electrode, indicating the inhibition of the electron transfer due to the presence of NC nanofibrils on the electrode surface. A similar situation was previously observed in the case of gold electrodes modified with self-assembled monolayers.[37]

The presence of the NC nanofibrils on the electrode was determined by EDX. Elemental peaks of N and O on the horizontal axis of the spectra corresponded to the NC octapeptide sequence forming the nanofibrils. These peaks were not present in the spectra of the bare gold electrode, as can be seen in Figure 4, displaying two EDX spectra pertaining to areas of the gold electrode surface with and without NC nanofibrils.

Electrochemical Response of Copper at the NC-Modified Gold Electrode. The influence of the accumulation time on the binding of copper to the NC-modified gold electrode was

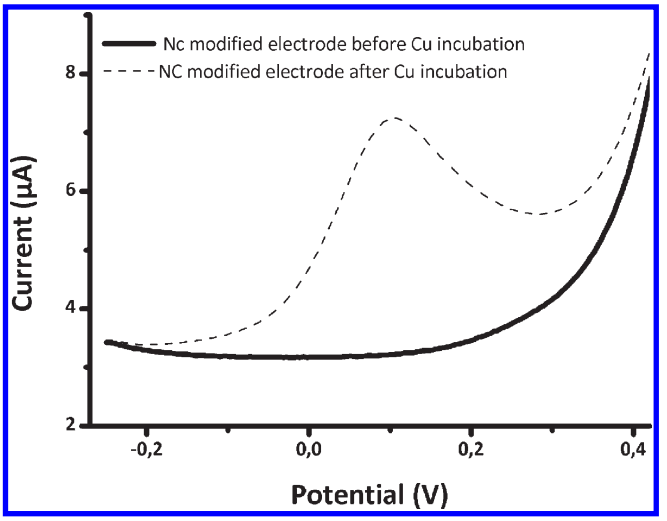

Figure 5. Square wave voltammograms of copper bound to NC nanofibrils immobilized on a screen-printed gold electrode with a 60-μM copper solution in 50-mM ammonium acetate (pH 6.8) for 2 min, applied potential vs Ag/AgCl.

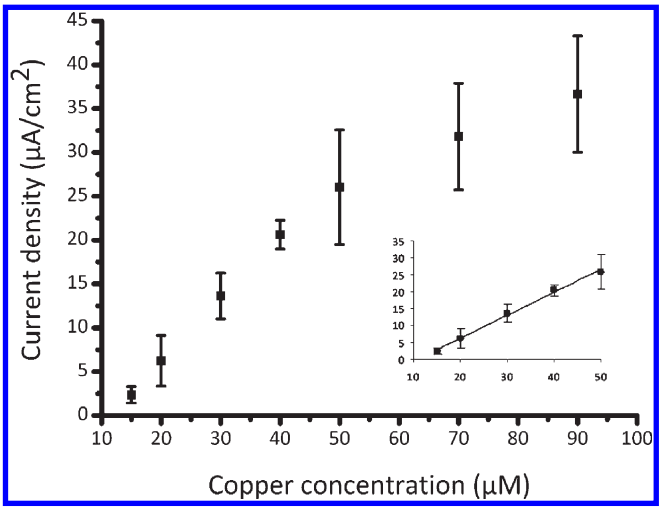

Figure 6. Calibration curve of the NC-modified screen-printed gold electrode for varying copper concentrations.

investigated. In order to find the optimal accumulation time, an NC-modified gold electrode was evaluated using a 45 μM copper solution. Previous investigations have demonstrated that longer incubation times lead to an increase in the detected current,[38,39] and similar results were obtained during the present study.

As expected, the response curve was dependent on the accumulation time (data not shown). The signal increased linearly with the preconcentration from 1 to 8 min reaching equilibrium at 10 min, suggesting a saturation of accumulated copper on the electrode surface. Previous studies have reported preconcentration times longer than 5 min.[38,40] For our purpose, a 2 min preconcentration time was efficient and was chosen in order to maintain a short experiment time. Obviously, this increased the detection limit, but depending on the application, a reasonable compromise should be established between the preconcentration time and the detected signal.

Once the preconcentration time was established, several solutions of copper in 50 mM ammonium acetate (pH 6.8) were

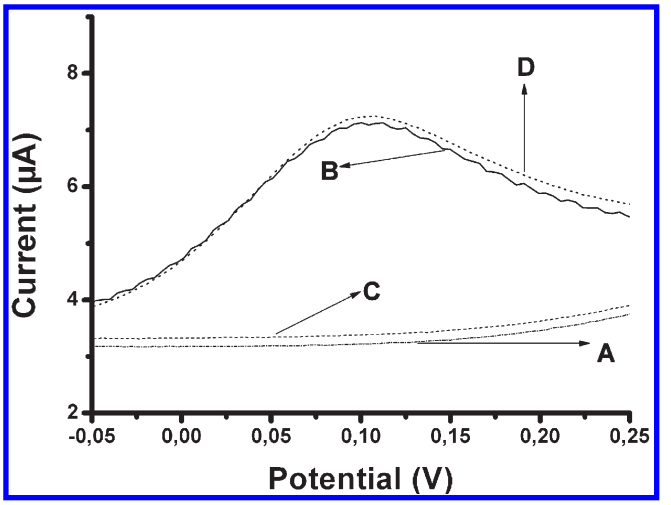

Figure 7. Square wave voltammogram of copper bound to NC-nanofibrils immobilized on a screen-printed gold electrode with a 60-μM copper solution in 50 mM ammonium acetate (pH 6.8) for 2 min. After the initial copper accumulation, the electrode surface was regenerated to a copper-free state. SWV signals before (A) and after (B) copper accumulation, regeneration to a copper-free state (C) and after a new copper accumulation. Applied potential vs Ag/AgCl.

used to evaluate the linear range and detection limit of the sensor. Buffers with pH values ranging from 5 to 9 were also evaluated, but the best results were obtained when using buffer with a pH value of 6.8 (data not shown). The use of ammonium acetate buffer with pH values around 6.8 for the determination of copper ions has been previously reported as a good choice.[20,41] The fact that the peptides used in our study have amidated ($-CONH_2$) C-termini avoid complexation of metal at this pH as could be expected when using peptides containing C-terminal carboxylates. For quantification purposes, square wave voltammogram (SWV) was used. Figure 5 shows these voltammograms before and after the accumulation of a 60 μM copper solution in 50 mM ammonium acetate (pH 6.8) for 2 min.

An increase in current was observed after immersion of the NC-modified electrode in the copper solution. Contrarily, a flat signal was observed for an NC-modified electrode where the copper preconcentration time was zero.

Figure 6 shows a calibration curve using the peak current value obtained during the SWV with the several copper concentrations. The sensor displayed a linear range up to a concentration of 50 μM copper (R^2=0.996) and a sensitivity of 0.68 μA cm^{-2} μM^{-1}.

Previous work has reported the use of oligopeptides modified electrode surfaces and ISE for the electrochemical detection of copper with detection limits in the nanomolar range.[17,20,26−29,42,43] However, in some cases the transducer surface needs a pretreatment process that could take a couple of hours, to allow the attachment of the oligopeptides.[28,42] Our approach allows a direct surface modification of the gold electrode takes without the need of pretreatment steps. Besides, the biorecognition element is a 1D self-assembled nanomaterial. 1D nanomaterials displays a large surface-to-volume ratio and relatively short diffusion length, properties that could enhance the electrochemical properties.[44] Additionally, the nanofibrils composition could be tuned in order to allow the detection of more metal ions without losing the ability to self-assemble into a 1D nanostructure as previously demonstrated.[30]

To evaluate the possibility of reusing the developed sensor, the NC-modified sensor was regenerated to a copper free state as

explained in the Materials and Methods. Figure 7 presents the SWV signals obtained for the NC-modified gold electrode before (A) and after (B) copper accumulation as well as after the regeneration of the sensor to a copper-free state (C) and after a new copper accumulation (D). When comparing curves B and D, a small decrease of a few nA was observed for the copper signal for the same copper solution. These results support the possibility of reusing the sensor for several measurements.

Finally, it is necessary to add that a more detailed study to evaluate the behavior of the developed sensor in the presence of interference compounds such as Ca^{2+} or Mg^{2+} will be carried out. This will be requiring when measuring in samples such as water or biological fluids.

CONCLUSIONS

This article describes the use of self-assembled peptide nanofibers for the development of an electrochemical metal-ion biosensor for the detection of copper ions in solution. The NC-modified gold electrodes displayed a good coating of the metal surface giving rise to a complete insulation of transducer surface. The modified electrodes were characterized electrochemically and exhibited a linear response up to 50 μM for copper with a sensitivity of 0.68 $\mu Acm^{-2}\mu M^{-1}$. These characteristics of the developed sensor suggested that it could be utilized in environmental applications, e.g., copper detection in drinking water samples were the maximum amount of copper allowed is below 30 μM.

The fabricated sensor can be regenerated to a copper-free state permitting reuse without a significant decrease of the signal. These self-assembled peptide nanofibrils are thus promising materials for the detection of metal ions, and a next step will be the possibility of tuning the peptide sequence to interact with a specific metal without losing the ability to self-assemble into nanofibrillar structures as well as evaluating the effect of possible interfering metal ions.

ASSOCIATED CONTENT

Supporting Information. Cyclic voltammograms of graphite, platinum, and gold electrodes before and after modification with self-assembled fibrils. This material is available free of charge via the Internet at http://pubs.acs.org.

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REFERENCES

(1) Boncheva, M.; Whitesides, G. M. *Mater. Res. Bull.* **2005**, *30*, 736–742.
(2) Zhang, S. G. *Nat. Biotechnol.* **2003**, *21*, 1171–1178.
(3) Brea, R. J.; Reiriz, C.; Granja, J. R. *Chem. Soc. Rev.* **2010**, *39*, 1448–1456.
(4) Scanlon, S.; Aggeli, A. *Nano Today* **2008**, *3*, 22–30.
(5) Yan, X. H.; Zhu, P. L.; Li, J. B. *Chem. Soc. Rev.* **2010**, *39*, 1877–1890.
(6) Zanuy, D.; Nussinov, R.; Aleman, C. *Phys. Biol.* **2006**, *3*, S80–S90.
(7) Chun, A.; Morales, J.; Webster, T.; Fenniri, H. In *Nanotechnology in Biology and Medicine. Methods, Devices and Applications*; Vo-Dinh, T., Eds.; CRC Press: Boca Raton, FL, 2007; pp 1−2.
(8) de la Rica, R.; Matsui, H. *Chem. Soc. Rev.* **2010**, *39*, 3499–3509.
(9) de la Rica, R.; Mendoza, E.; Matsui, H. *Small* **2010**, *6*, 1753–1756.
(10) MacPhee, C. E.; Woolfson, D. N. *Curr. Opin. Solid State Mater. Sci.* **2004**, *8*, 141–49.
(11) Pepe-Mooney, B. J.; Fairman, R. *Curr. Opin. Struct. Biol.* **2009**, *19*, 483–494.
(12) Zhao, X. J.; Zhang, S. G. . In *Polymers for Regenerative Medicine*; Springer-Verlag Berlin: Berlin, 2006; pp 145−170.
(13) Hauser, C. A. E.; Zhang, S. G. *Chem. Soc. Rev.* **2010**, *39*, 2780–2790.
(14) Toksoz, S.; Guler, M. O. *Nano Today* **2009**, *4*, 458–469.
(15) Papanikolopoulou, K.; Schoehn, G.; Forge, V.; Forsyth, V. T.; Riekel, C.; Hernandez, J. F.; Ruigrok, R. W. H.; Mitraki, A. *J. Biol. Chem.* **2005**, *280*, 2481–2490.
(16) Gooding, J. J.; Erokihn, P.; Losic, D.; Yang, W.; Policarpio, V.; Liu, J.; Ho, F. M.; Situmorang, M.; Hibbert, D. B.; Shapter, J. G. *Anal. Sci.* **2001**, *17*, 3–9.
(17) Chow, E.; Gooding, J. J. *Electroanalysis* **2006**, *18*, 1437–1448.
(18) Huster, D.; Lutsenko, S. *Mol. BioSyst.* **2007**, *3*, 816–824.
(19) Stern, B. R.; Solioz, M.; Krewski, D.; Aggett, P.; Aw, T. C.; Baker, S.; Crump, K.; Dourson, M.; Haber, L.; Hertzberg, R.; Keen, C.; Meek, B.; Rudenko, L.; Schoeny, R.; Slob, W.; Starr, T. *J. Toxicol. Environ. Health, Part B* **2007**, *10*, 157–222.
(20) Chow, E.; Wong, E. L. S.; Bocking, T.; Nguyen, Q. T.; Hibbert, D. B.; Gooding, J. J. *Sens. Actuators, B* **2005**, *111*, 540–548.
(21) Ghaedi, M.; Ahmadi, F.; Shokrollahi, A. *J. Hazard. Mater.* **2007**, *142*, 272–278.
(22) Jakubeniene, M.; Zakaras, A.; Minkuviene, Z. N.; Benoshys, A. *Forensic Sci. Int.* **2006**, *161*, 36–40.
(23) Knecht, M. R.; Sethi, M. *Anal. Bioanal. Chem.* **2009**, *394*, 33–46.
(24) Castillo, J.; Gaspar, S.; Leth, S.; Niculescu, M.; Mortari, A.; Bontidean, I.; Soukharev, V.; Dorneanu, S. A.; Ryabov, A. D.; Csoregi, E. *Sens. Actuators, B* **2004**, *102*, 179–194.
(25) Nie, Z.; Nijhuis, C. A.; Gong, J.; Chen, X.; Kumachev, A.; Martinez, A. W.; Narovlyansky, M.; Whitesides, G. M. *Lab Chip* **2010**, *10*, 477–483.
(26) Gooding, J. J.; Hibbert, D. B.; Yang, W. *Sensors* **2001**, 1.
(27) Rachou, J.; Gagnon, C.; Sauve, S. *Environ. Chem.* **2007**, *4*, 90–97.
(28) Yang, W.; Chow, E.; Willett, G. D.; Hibbert, D. B.; Gooding, J. J. *Analyst* **2003**, *128*, 712–718.
(29) Zeng, Z. L.; Menzies, N. W.; Kerven, G. *Electroanalysis* **2005**, *17*, 912–914.
(30) Kasotakis, E.; Mossou, E.; Adler-Abramovich, L.; Mitchell, E. P.; Forsyth, V. T.; Gazit, E.; Mitraki, A. *Biopolymers* **2009**, *92*, 164–172.
(31) Tamamis, P.; Kasotakis, E.; Mitraki, A.; Archontis, G. *J. Phys. Chem. B* **2009**, *113*, 15639–15647.
(32) Eckermann, A. L.; Feld, D. J.; Shaw, J. A.; Meade, T. J. *Coord. Chem. Rev.* **2010**, *254*, 1769–1802.
(33) Nuzzo, R. G.; Fusco, F. A.; Allara, D. L. *J. Am. Chem. Soc.* **1987**, *109*, 2358–2368.
(34) Dinca, V.; Kasotakis, E.; Catherine, J.; Mourka, A.; Ranella, A.; Ovsianikov, A.; Chichkov, B. N.; Farsari, M.; Mitraki, A.; Fotakis, C. *Nano Lett.* **2008**, *8*, 538–543.
(35) Dinca, V.; Kasotakis, E.; Mourka, A.; Ranella, A.; Farsari, M.; Mitraki, A.; Fotakis, C. *Phys. Status Solidi C* **2008**, *5*, 3576–3579.
(36) Wilke, N.; Baruzzi, A. M. *J. Electroanal. Chem.* **2002**, *537*, 67–76.
(37) Niu, L. M.; Luo, H. Q.; Li, N. B.; Song, L. *J. Anal. Chem.* **2007**, *62*, 470–474.

(38) Liu, A. C.; Chen, D. C.; Lin, C. C.; Chou, H. H.; Chen, C. H. *Anal. Chem.* **1999**, *71*, 1549–1552.
(39) Zeng, B. Z.; Ding, X. G.; Zhao, F. Q.; Yang, Y. X. *Anal. Lett.* **2002**, *35*, 2245–2258.
(40) Yang, W. R.; Gooding, J. J.; Hibbert, D. B. *Analyst* **2001**, *126*, 1573–1577.
(41) Zeng, B. Z.; Ding, X. G.; Zhao, F. Q. *Electroanalysis* **2002**, *14*, 651–656.
(42) Bi, X.; Agarwal, A.; Yang, K.-L. *Biosens. Bioelectron.* **2009**, *24*, 3248–3251.
(43) Bi, X. Y.; Wong, W. L.; Ji, W. J.; Agarwal, A.; Balasubramanian, N.; Yang, K. L. *Biosens. Bioelectron.* **2008**, *23*, 1442–1448.
(44) Wang, J. *Analyst* **2005**, *130*, 421–426.