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Research Article

Manipulation of self-assembly amyloid peptide nanotubes by dielectrophoresis

Self-assembled amyloid peptide nanotubes (SAPNT) were manipulated and immobilized using dielectrophoresis. Micro-patterned electrodes of Au were fabricated by photolithography and lifted off on a silicon dioxide layer. SAPNT were manipulated by adjusting the amplitude and frequency of the applied voltage. The immobilized SAPNT were evaluated by SEM and atomic force microscopy. The conductivity of the immobilized SAPNT was studied by *I–V* characterization, for both single SAPNT and bundles. This work illustrates a way to manipulate and integrate biological nanostructures into novel bio-nanoassemblies with concrete applications, such as field-effect transistors, microprobes, microarrays, and biosensing devices.

Keywords:
Bionanotechnology / Dielectrophoresis / Peptide nanotubes / Self-assembly
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1 Introduction

The elegant self-organization of bionanostructures into well-defined functional architectures found in nature has been an invaluable source of inspiration for researchers. The molecules of life, proteins, lipids, DNA, RNA, vitamins, *etc.*, as well as the structures and forms that these molecules assume serve as rich sources of ideas for scientists or engineers who are interested in developing bio-inspired materials for innovations in biomedical fields [1]. In nature, molecular self-assembly is a process by which complex three-dimensional structures with well-defined functions are constructed starting from simple building blocks such as proteins or peptides [2, 3]. Peptide materials are non-toxic and may be used in the biological and medical contexts [4]. As building blocks peptides present the advantages of chemical diversity, flexibility, biocompatibility, and stability, which make them excellent candidates to be used in nanotechnology and bionanotechnology processes [5–9].

The use of cyclic peptides for the design and synthesis of biological nanotubes was first reported more than 12 years ago, when tubular nanostructures with internal diameters of 7–8 Å were fabricated and reported [10, 11]. The use of peptide nanotubes as tools with a high potential for applications in bionanotechnology has been highlighted in several reports [12–18].

The amyloid peptide used in this study is the diphenylalanine peptide. This short aromatic peptide is the core recognition element of the Alzheimer's disease β-amyloid peptide. Its single crystal structure was previously studied by X-ray powder diffraction [19]. It was reported to self-assemble by dilution of a concentrated solution of the dipeptide in water [20]. The diphenylalanine peptide forms ordered long and stiff nanotubes with internal diameters of about 20 nm, external diameters ranging from 80 to a few hundred nanometres, and length in the micrometer range [21]. A previous study showed that the nanotubes formed by this aromatic dipeptide have a remarkable thermal and chemical stability [22]. Additional to these characteristics the amyloid peptide nanotubes also show an extraordinary mechanical strength with a Young's modulus of ~19 GPa and a point stiffness of 160 N/m [23].

All these properties make self-assembled amyloid peptide nanotubes (SAPNT) very attractive building blocks for use in various nanotechnology applications. Amperometric biosensors were recently developed for the detection of compounds of biomedical importance such as glucose, ethanol, and hydrogen peroxide using the amyloid diphenylalanine motif as a biorecognition element [24–26]. Metal nanowires were fabricated by reducing ionic silver within the nanotubes followed by enzymatic degradation of the peptide backbone. As a result silver nanowires were produced [20]. Self-assembling amyloid protein fibres have also been used to build nanowires by controlled selective reductive deposition of gold and silver from salts. In this way silver and gold nanowires ≈100 nm wide were obtained [27]. Nanowire-based nanoelectronics devices are believed to bring revolutionary advances in biomedical sciences.

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Abbreviations: AFM, atomic force microscopy; **DEP,** dielectrophoresis; **SAPNT,** self-assembled amyloid peptide nanotubes

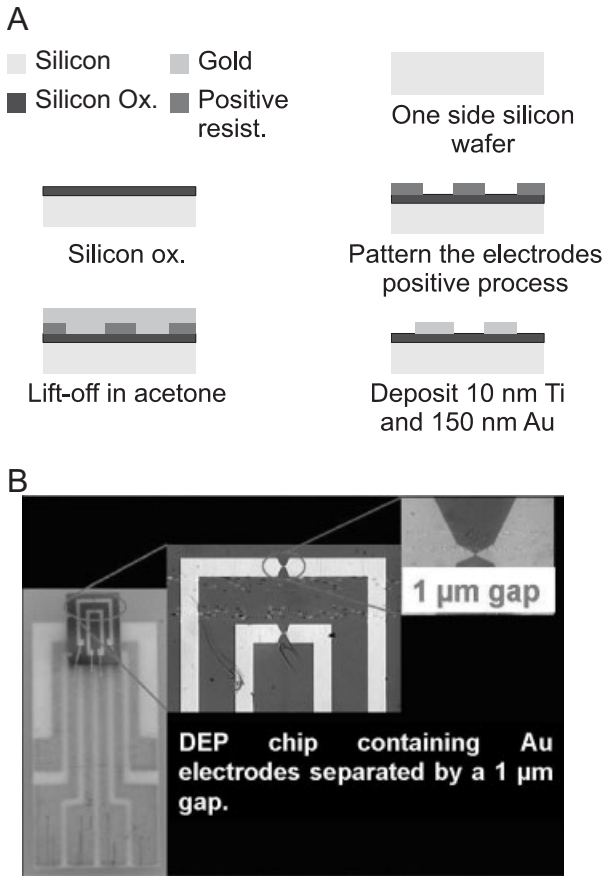

Figure 1. (A) Microchip fabrication process by optical lithography on a silicon wafer as reported by Dimaki and Boggild [32]. (B) Final DEP microchip structure showing the gap between the gold microelectrodes.

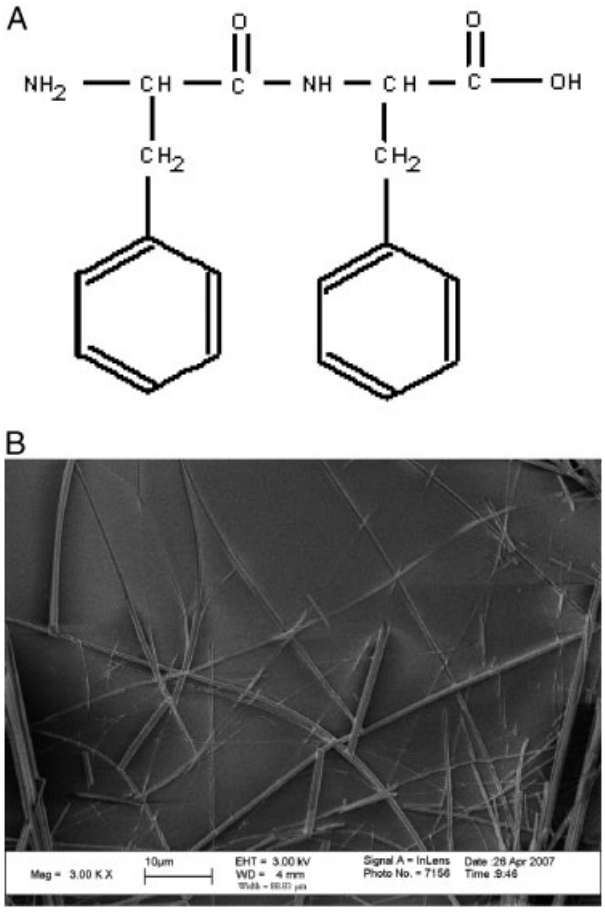

Figure 2. (A) Molecular structure of the diphenylalanine peptide. (B) SEM image of the diphenylalanine peptide nanotubes lying on a silicon oxide surface.

The integration of functionalized nanofibres and nanotubes with electronic devices will result in analytical tools sensitive enough to analyse key biological and chemical species in a rapid and direct way in order to uncover and diagnose diseases [28–31]. All these applications accentuate the necessity to find techniques to control the manipulation of these bionanostructures. In this work we present the fabrication and use of a dielectrophoresis (DEP) microchip that allows the manipulation of the amyloid dipeptide nanotubes in a controlled way. This article constitutes the first step in the integration of these building blocks into biosensing devices such as biosensors and field-effect transistor microchips for biomedical applications.

2 Materials and methods

2.1 DEP microchip fabrication

The microchip was fabricated by optical lithography on a silicon wafer following a protocol previously described [32]. Briefly, SiO_2 was grown on top of a silicon wafer as an insulating layer. A 1.5 µm resist layer was spun on top of the oxide and a positive photolithography process was used in order to pattern the electrodes on the oxide. After development of the resistance 10 nm of titanium and 150 nm of gold were deposited on the wafer and a lift-off process using acetone was carried out to define the electrodes. The titanium layer is necessary to enhance the adhesion between the gold and the silicon oxide layer. Figure 1A illustrates the fabrication steps. The final DEP microchip and the separation gap between the gold microelectrodes is shown in Fig. 1B.

2.2 Peptide stock solution preparation

Diphenylalanine peptide was purchased from Bachem (Cat. no. G-2925, Germany). Fresh stock solutions were prepared by dissolving the lyophilized form of the peptide in 1,1,1,3,3,3-hexafluoro-2-propanol (Sigma Aldrich) at a final concentration of 100 mg/mL. Fresh solutions were prepared before each experiment.

2.3 DEP experiments

Peptide stock solution was diluted in distilled water to a final concentration of 2 mg/mL. An aliquot of 2 mg/mL peptide solution was placed on top of the microelectrodes. The alternating current voltage was then turned on and the different parameters were applied: frequency, potential magnitude, and time. Voltage amplitudes from 1 to 10 V peak–peak, frequencies from 0.1 to 10 MHz, and times ranging from 30 s to 5 min were applied on the electrodes for the DEP experiment. After the chosen time was finished the voltage was turned off and the excess of solvent was removed from the chip by using a stream of nitrogen.

2.4 SEM imaging of the amyloid peptide nanotubes

All SEM images were carried out with a LEO 1550 Scanning Electron Microscope with EDX. Previous to the SEM imaging the amyloid peptide nanotubes were covered with a gold layer using a Hummer gold sputtering system.

2.5 Atomic force microscopy imaging of the immobilized peptide nanotubes

All atomic force microscopy (AFM) images were carried out with a Veeco CP-II Scanning Probe Microscope (Veeco Systems). Images of peptide nanotubes were obtained in AFM taping mode in air using an ElectriTap 300 probe (Budget Sensors).

2.6 I–V curve

For the *I–V* curve a low-noise current pre-amplifier Model SR570 (Stanford Research Systems) and a BNC-211 adapter (National Instruments) for data acquisition were used.

3 Results and discussion

3.1 Fabrication of amyloid peptide nanotubes

Amyloid peptide nanotubes were obtained by dissolving aliquots of a concentrated diphenylalanine peptide stock solution in water. The chemical structure of the peptide used in this work is shown in Fig. 2A. The fabricated peptide nanotubes were imaged using SEM. Initial analysis showed the formation of long and thick peptide nanotube bundles (Fig. 2B). In order to obtain more separate nanotube bundles and even individual nanotubes a lesser concentrated solution, 0.5 mg/mL, was prepared.

3.2 Manipulation and immobilization of the self-assembly amyloid peptide nanotubes

DEP occurs when a polarizable particle is suspended in an inhomogeneous electric field, so that the electrical forces induced on the charges on each half of the dipole are different [33]. In this way nanowires can be oriented and connected to electrodes where mechanical, electrical, and

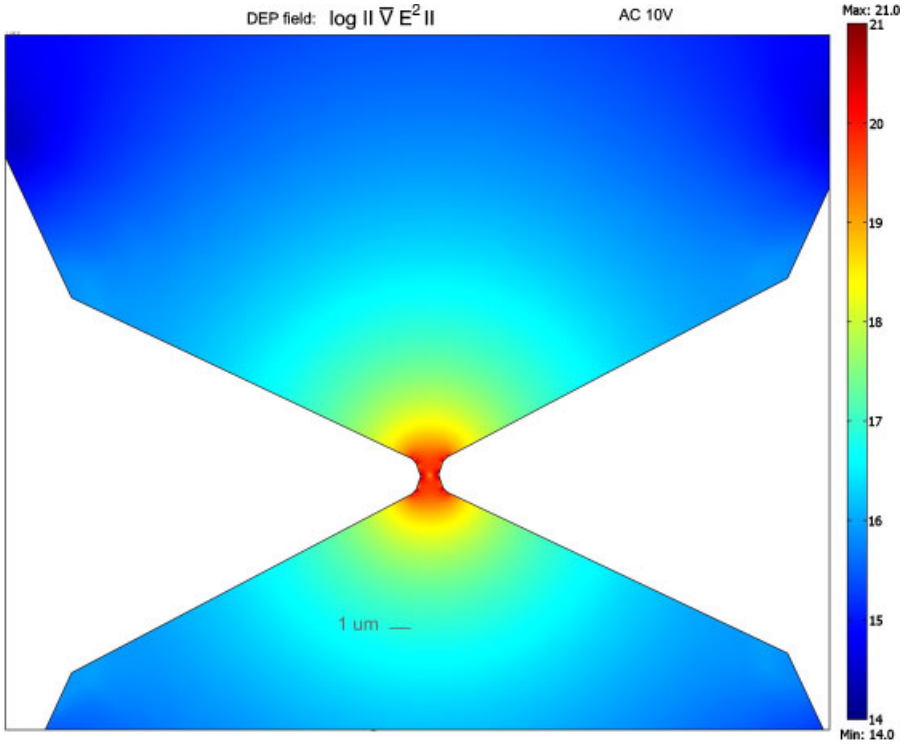

Figure 3. DEP field simulation in the fabricated gold microelectrodes using Comsol Multiphysics software. The gap between the electrodes is 1 μm and the applied potential corresponded to a value of 10 V peak–peak.

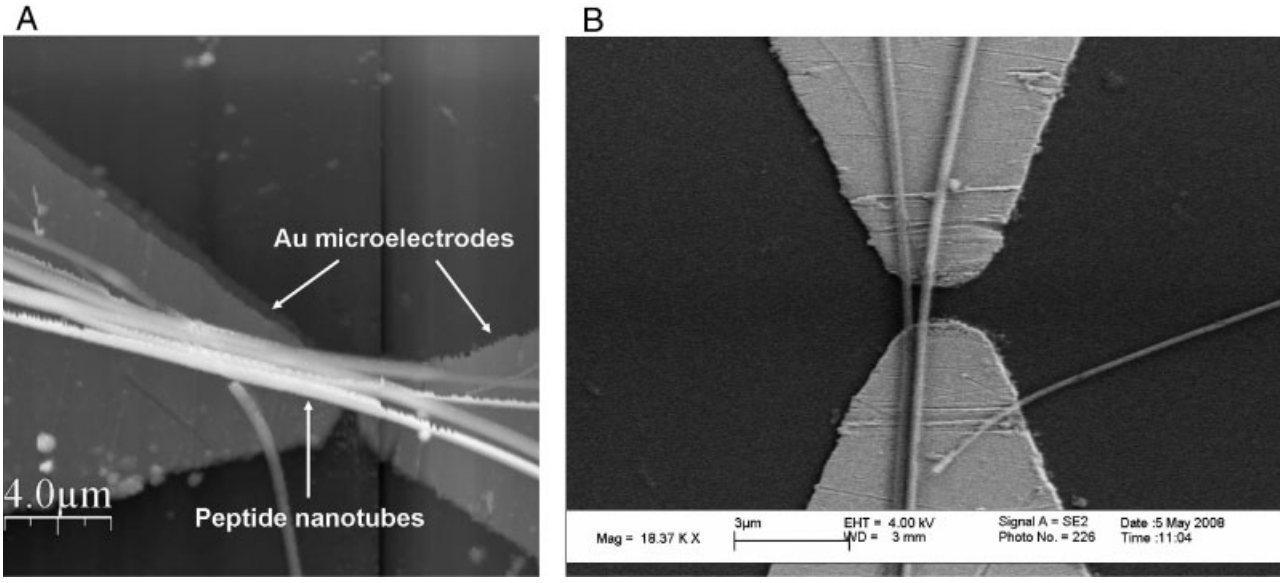

Figure 4. (A) AFM image of the SAPNT bundles immobilized on Au electrodes using DEP. (B) SEM image of two amyloid peptide nanotubes immobilized on top of gold microelectrodes.

properties such as conductivity, permittivity, and polarizability can be investigated.

DEP has been used for the analysis and separation of a variety of biological particles such as cells and DNA [34–38].

Previous to the DEP experiments, a two-dimensional simulation by Comsol Multiphysics software was used to simulate the electrical field on the fabricated microelectrodes with a 1 μm gap. For the simulation we used the permittivity of water, $\varepsilon_m = 80$, and a value of 10 V peak–peak was used as the applied potential. The result of the simulation is shown in Fig. 3. The maximum of the DEP field, indicated by the red colour is situated at the tip of the gold microelectrodes as anticipated. In this way we expected the amyloid peptide nanotubes to be trapped between the two tips of the gold microelectrodes. Owing to the fact that the electrical properties of the nanotubes used in this work have not previously been studied the dielectrical constant of them is unknown. For this reason we did not include the nanotubes in the simulation.

For the DEP experiments a drop of the peptide nanotube suspension (~5 μL) with a concentration of 2 mg/mL was applied on top of the chip with a micropipette. The DEP microchip was connected to the function generator through a custom-made holder.

After this the frequency generator was switched on. After 5 min, the generator was turned off and the drop was blown off the surface with a nitrogen stream. For the DEP experiments alternating current voltage with frequency values from 0.1 to 10 MHz, amplitude from 1 to 10 V for times ranging between 30 s and 5 min were evaluated. Different parameter combinations were tried in order to get the optimal values to manipulate the nanotubes. Normally the positive DEP response for particles shows a broad maximum as a function of frequency rather than a sharp maximum. However, in our case from all the experiments performed using the different frequency and voltage values, only when an alternating current voltage of 10 V with a frequency of 1 MHz for 5 min was applied, a number of amyloid peptide bundles were successfully deposited on top of the microelectrodes.

A typical result of the immobilization of amyloid peptide nanotubes bundles onto a gold microelectrode is shown in Fig. 4A and B. These show an AFM and an SEM image of amyloid peptide nanotubes respectively, connecting two gold microelectrodes. The nanotubes are aligned along the two-microelectrode tips after the DEP experiment.

An important goal in our work was the immobilization of a single amyloid peptide nanotube. In order to do this, it was necessary to prepare a more dilute, 0.5 mg/mL, peptide solution. In this way more separate peptide nanotubes were obtained. Single amyloid peptide nanotubes were previously imaged using AFM (Fig. 5). The topography line scan in Fig. 5A shows a smooth peptide nanotube surface without any large features. The height of this peptide nanotube above the surface can be measured from the topography line scan in Fig. 5B, and it was found to be 83 ± 5 nm.

The phase scan of the same peptide nanotube is shown in Fig. 6B. The phase scan contains a dip in the centre, which can clearly be seen in the phase line scan in Fig. 6B. This dip was found to be a characteristic of the phase scans and illustrated the hollow nature of the peptide nanotubes.

After repeating the steps mentioned before for the immobilization of peptide nanotube bundles a single amyloid peptide nanotube was manipulated and deposited on top of the chip microelectrodes, as presented in Fig. 7.

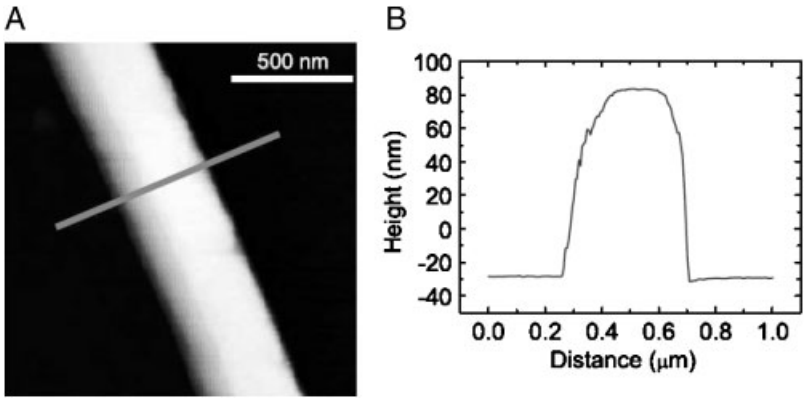

Figure 5. Topography and line scan of a peptide nanotube lying on a silicon oxide surface: (A) AFM topography image of a single amyloid peptide nanotube. (B) The line scan corresponding to the blue line in (A).

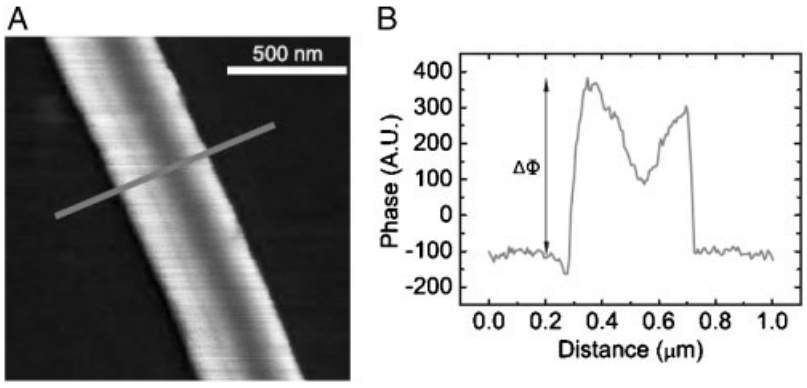

Figure 6. Phase image and line scan of a peptide nanotube lying on a silicon oxide surface: (A) Phase image of a peptide nanotube. (B) The line scan corresponding to the red line in (A). The measure phase shift, $\triangle\Phi$, is indicated on the line scan.

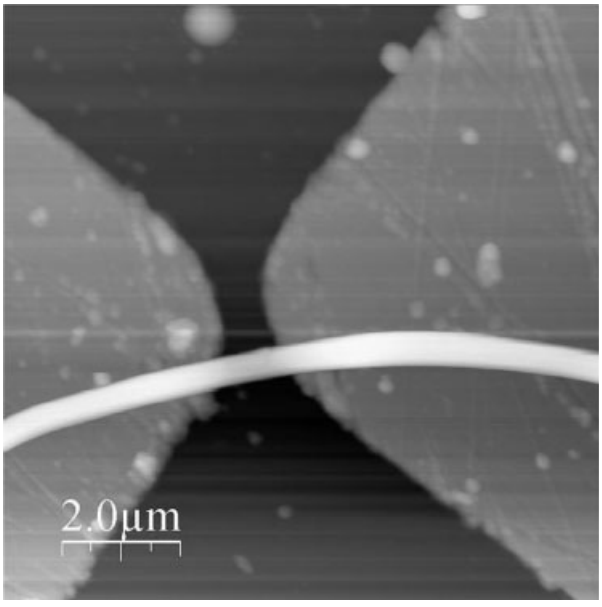

Figure 7. AFM image of a single amyloid peptide nanotube immobilized on top of gold microelectrodes using DEP.

3.3 I–V curve

To evaluate the electrical behaviour of the amyloid peptide nanotubes an *I–V* curve was plotted. Previously, binding of the nanotubes to the microelectrodes and bridging of the gap between the gold microelectrodes was confirmed by AFM. Passing current through this set-up allowed a reading of the current (*I*) and voltage (*V*), and the *I–V* curve for amyloid nanotube bundles was recorded. As shown in Fig. 8, the immobilized SAPNT bundles presented a very low conductivity; this behaviour confirms the insulator properties of this kind of biological nanotubes as it was expected. The current transmitted through the immobilized nanotubes after an applied potential of 0–3 V was in the pA range, (black line). The jump from around 0 A to approximately 1.0×10^{-12} A is due to the offset voltage when potential is applied at the beginning of the experiment. The low conductivity of the SAPNT was confirmed when the *I–V* curve was plotted for a single SAPNT bridging the gap between the two gold microelectrodes (red line). In this case the conductivity was even lower than that for the immobilized SAPNT bundles as it was expected. As a control experiment, and to be sure that the current obtained was the one passing through the peptide nanotubes, an *I–V* curve using the same type of DEP chip but without any nanotube immobilized on top was done. In this case a flat line showing zero conductivity was obtained (blue line). Surprisingly the nanotubes were still present on the microelectrodes after several potential cycles from 0 to 3 V were applied to them. This is an indication of the resistance of the nanotubes to high voltages and opens new possibilities for applications in nanoelectronic devices.

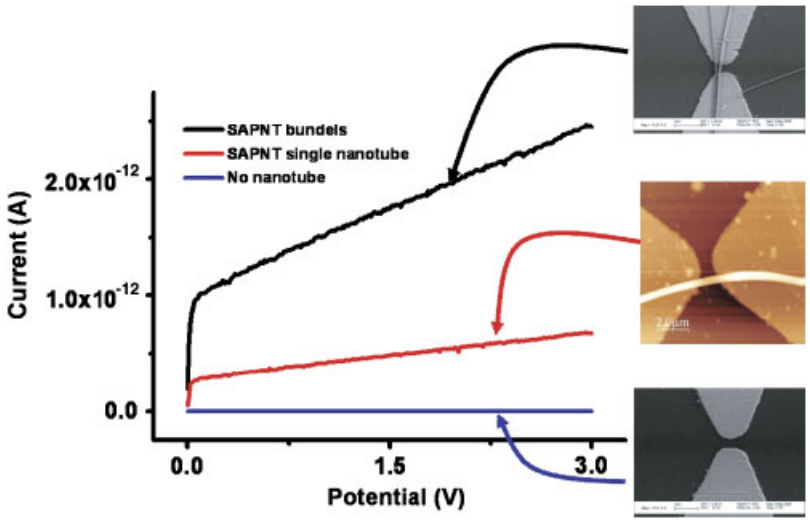

Figure 8. *I–V* curve. Amyloid peptide nanotube bundles (black line) and single amyloid peptide nanotube (red line) bridging the gap between two microelectrodes exhibit linear *I–V* curves, demonstrating ohmic conductivity with very high resistance. Control experiment, *I–V* curve for the empty holder, blue line.

4 Concluding remarks

A DEP microchip was fabricated by optical lithography and lift-off. Amyloid peptide nanotube bundles and even single nanotubes were manipulated and immobilized in a controlled way using DEP on top of gold microelectrodes. The immobilized nanotubes were imaged by AFM. Their conductivity was studied, showing that these bionanostructures present a low ohmic conductivity when a potential is applied. This finding suggests the necessity to functionalize the nanotubes with metal nanoparticles in order to reduce their high resistance and increase their conductivity. No data commenting on the electrical properties of these kinds of nanotubes have been previously reported. This work represents the first step in the integration of these bionanostructures in biosensing and bioelectronic devices such as biosensors and field-effect transistor microchips for the detection of compounds of biomedical relevance. The integration of microfluidics with the developed DEP chip is suggested as a way to improve the orientation and immobilization of the nanotubes before reaching the microelectrodes.

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The authors have declared no conflict of interest.

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