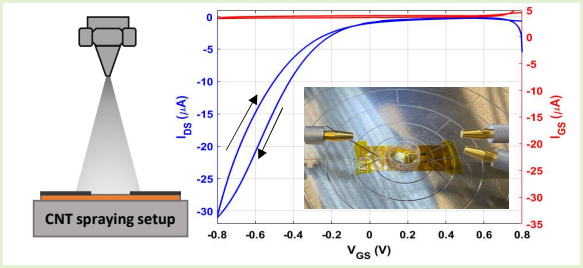
Optimization of the spray-deposited carbon nanotube semiconducting channel for electrolyte-gated field-effect transistor-based biosensing applications

Bajramshahe Shkodra, Mattia Petrelli, *Student Member, IEEE*, Martina Aurora Costa Angeli, *Member, IEEE*, AKM Sarwar Inam, Paolo Lugli, *Fellow, IEEE*, and Luisa Petti, *Member, IEEE*

***Abstract*— Electrochemical biosensors are widely investigated as they represent attractive analytical tools for detection of a broad range of bio-molecules, thanks to their simplicity, high sensitivity and short response time. Especially, biosensors employing an electrolyte-gated field-effect transistors (EG-FETs) as electrochemical transduction element have gained increasing interest, due to the signal amplification and the intrinsic low voltage range of operation. In this work we report the fabrication of flexible EG-FETs using spray-deposited semiconducting carbon nanotubes (CNTs), with a specific focus on the optimization of the CNT channel to optimize the performance of the resulting CNT-based EG-FET (EG-CNTFET). The transfer and the output characteristic of different devices with varying spraying parameters were tested, finding out that only devices with source-drain resistance of about $\leq$10 kΩ showed proper EG-CNTFET operation: for these devices we recorded a typical p-type behavior with an on–off ratio of 214 A/A up to 469 A/A (depending on number of the spray-deposited CNT layers). The fabricated EG-CNTFETs were functionalized with anti-spermidine antibodies to detect polyamine spermidine - a well-known chemical indicator of food quality. To ensure controlled immobilization and at the same time to preserve the electrical properties of the nanotubes, the spray-deposited films were modified with a bifunctional molecule, which attaches to the CNT via non-covalent $\pi - \pi$ interactions and leaves a free NHS-ester group for amide coupling of the antibodies. The fabricated EG-CNTFET-based immunosensors showed a linear detection range for spermidine from 10^{-3} to 10^{2} nM, with the sensitivities ranging from -1.03 to -2.45 μA/decade.**



***Index Terms*— CNTs, electrolyte-gated, flexible electronics, immunosensors, polyamines, transistors**

I. Introduction

IN RECENT YEARS, electrochemical biosensing methods have been widely investigated for application in several fields such as biomedicine and healthcare [1], wearable electronics [2], as well as food and beverage quality control [3], [4]. An extensive effort has been dedicated to the development of new classes of electrochemical biosensors, characterized by features such as low manufacturing costs, fast response time, high sensitivity and selectivity. In particular, biosensors using electrolyte-gated field-effect transistors (EG-FETs) as an electrochemical transduction platform have attracted significant interest in recent years, thanks to their ability to detect a wide range of bio-molecules with high sensitivity and selectivity, due to their intrinsic signal amplification [5], as well as to the possibility they offer to choose between different bio-recognition elements [6]. A typical planar EG-FET architecture consists of three metallic electrodes, namely source, drain, and gate. The source and drain electrodes are connected by a semiconducting channel, while all three metallic electrodes and the semiconducting channel are covered by a water-based electrolyte, which acts as gate dielectric [7]. Upon polarization of the gate, a rearrangement of the ions in the electrolyte occurs, resulting in the formation of the so-called electric double layers (EDLs), characterized by high specific capacitance values as compared to conventional metal oxide FETs ($> 1\,\mu F/cm^2$), which allow the operation of the EG-FET in the voltage range $< |2|V$ [5].

EG-FETs are typically fabricated using semiconductors such

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The authors are all with the Sensing Technologies Lab, Faculty of Science and Technology, Free University of Bozen-Bolzano, Piazza Università, 1, 39100, Bolzano, Italy (email: MCostaAngeli@unibz.it).

as metal-oxides [8], organic polymers [9], graphene [1], [10], as well as carbon nanotubes (CNTs) [11], [12].

The operation mechanism of EG-FET-based biosensors relies on the attachment of the analyte of interest to its specific bio-recognition element, which in turn causes a flow of carriers (holes/electrons) in the channel that either accumulate or deplete, leading to a change in the conductance of the semiconducting channel. As this resulting change in conductance depends on the concentration of the analyte, EG-FETs can be used for biosensing. To achieve good performance of EG-FET-based biosensors, coupling favourable properties of the active semiconducting channels and of the bio-recognition elements is required.

Recently, the demand for low-cost manufacturing processes of EG-FET-based biosensors has made solution-processed semiconducting CNTs a promising choice for the semiconducting channel. In fact, semiconducting CNTs offer carrier mobility as high as 100 $cm^2V^{-1}s^{-1}$ [13], large surface-to-volume ratio, chemical and electrochemical stability, simple fabrication process, as well as compatibility with a wide range of substrates from rigid wafers to flexible, self-standing foils [14], [15], which are all features of extreme interest to EG-FETs and biosensors in general. A further advantage of CNTs, is that the CNT semiconducting channel can be functionalized with a specific bio-recognition element to achieve a selective response toward the analyte of interest, [12], [16], [17]. Examples of carbon nanotube-based EG-FETs (EG-CNTFETs) used for biosensing include detection of bio-molecules [16], [18], cancer cells [19], real-time monitoring of cellular cultures [20] as well as different ions [12], [21].

In a recent study, we developed an EG-CNTFET-based biosensor for spermidine detection [22], presented at IEEE FLEPS 2021. Spermidine is part of the polyamines family and it is abundantly present in living cells [23]. In presence of other polyamines such as putrescine and cadaverine, spermidine may react with nitrite (present in food) to produce nitrosamines, a very well-known carcinogenic compounds [23], therefore its detection is of great importance for human health and could also be used as a chemical indicator of food quality. In general, biosensors are an excellent choice for rapid and efficient detection of allergens, pathogens, and toxicants, present in the food products.

Here we have focused our efforts on the investigation of the effect of the CNT network – made of spray-deposited semiconducting single-walled CNTs (SWCNTs) – on the performance of the EG-CNTFETs. Understanding the correlation between the properties of the semiconducting CNT channel and the EG-CNTFET biosensing performance is essential, an extremely careful optimization of the fabrication processes is higly required. Hence, in this work several spray-deposited parameters, with a particular focus on the CNTs number of layers, were optimize to achieve proper EG-CNTFET operation (i.e., it was possible to observe the modulation of the CNT channel conductivity through the gate voltage). Furthermore, the effect of different spray-deposited CNT layers in the performance of EG-CNTFET-based biosensor for spermidine detection has been investigated. The so-realized EG-CNTFET-based biosensors were used to determine the concentration of spermidine in a range of 10^{-3} to 10^{2} nM, reaching sensitivities ranging from -1.03 to -2.45 μA/decade depending on spray deposited semiconducting CNTs layers.

II. Methods

A. EG-CNTFET fabrication

The EG-FET layout consists of gold interdigitated electrodes (IDEs) for the source and the drain (channel length L = 50 μm, channel width W = 57 mm) and a planar gold gate electrode (Figure 1a), whose area was designed to be 4x the active area of the IDEs, as the increased area of the gate is proved to enhance the properties of planar EG-CNTFETs [24]. The advantage of using a planar gate is related to the straightforward fabrication process, as well as the facility in measurements since no external reference electrode is required. A single step of standard negative photolithography was used to pattern source, drain, and gate electrodes, followed by thermal evaporation of 10 nm of chromium as adhesion layer and of 50 nm of gold and subsequent lift-off. The CNTs were prepared for the spray deposition process at ambient conditions, in the form of a water-based solution, in which 0.05%wt CNTs (single walled, 95% semiconducting, Merck) were dispersed using 0.5%wt sodium carboxymethyl cellulose (CMC) (Merck) as surfactant. CMC was chosen as surfactant as it has been already demonstrated to be an effective dispersion reagent to achieve homogeneous well-dispersed semiconducting CNTs in aqueous medium, while preserving the intrinsic properties of the nanotubes [25]. The cellulose chains interact with the hydrophobic CNT surface stabilized by Van der Waals interaction while the hydrophilic carboxymethyl groups stabilize the dispersion in water due to hydrolysis [26].

To enhance the dispersion of the CNTs and further prevent the formation of bundles (i.e., agglomerates of CNTs that can severely hinder the performance of the devices [27]), the solution was treated using a horn sonicator (Fisherbrand™ FB-505), alternating 50% and 30% power 5 minutes cycles, for a total sonication time of 25 minutes. As a final step, the solution was centrifugated (Thermo Scientific™ SL 16, equipped with a F15-6 rotor) at 13000 rpm for 100 min in line with what reported in [28], [29]. To achieve homogeneous spray-deposited films (as can be observed in Figure S1) and to ensure semiconducting behavior of the nanotubes, the solution was diluted 1:30 in 1.3 mM CMC. Before spraying, the devices were rinsed with isopropyl alcohol (IPA) followed by DI-water, and finally dried using compressed air. Afterwards, they were treated by means of oxygen plasma for 1 min at 100 W, to increase the contact angle of the surface.

The spray deposition was carried out by means of an automated system equipped with an industrial air atomizing spray valve (Nordson EFD, USA) (Figure 1b). Several parameters were optimized to achieve the desired properties of the spray-deposited CNT films: the nozzle to substrate distance was fixed at 5 cm, the pressure of the atomizing airflow and of the material feed were kept at 0.5 and 0.2 bars, respectively. The speed of the moving arm was set at 150 mm/s, and the substrates were heated up at 70°C.

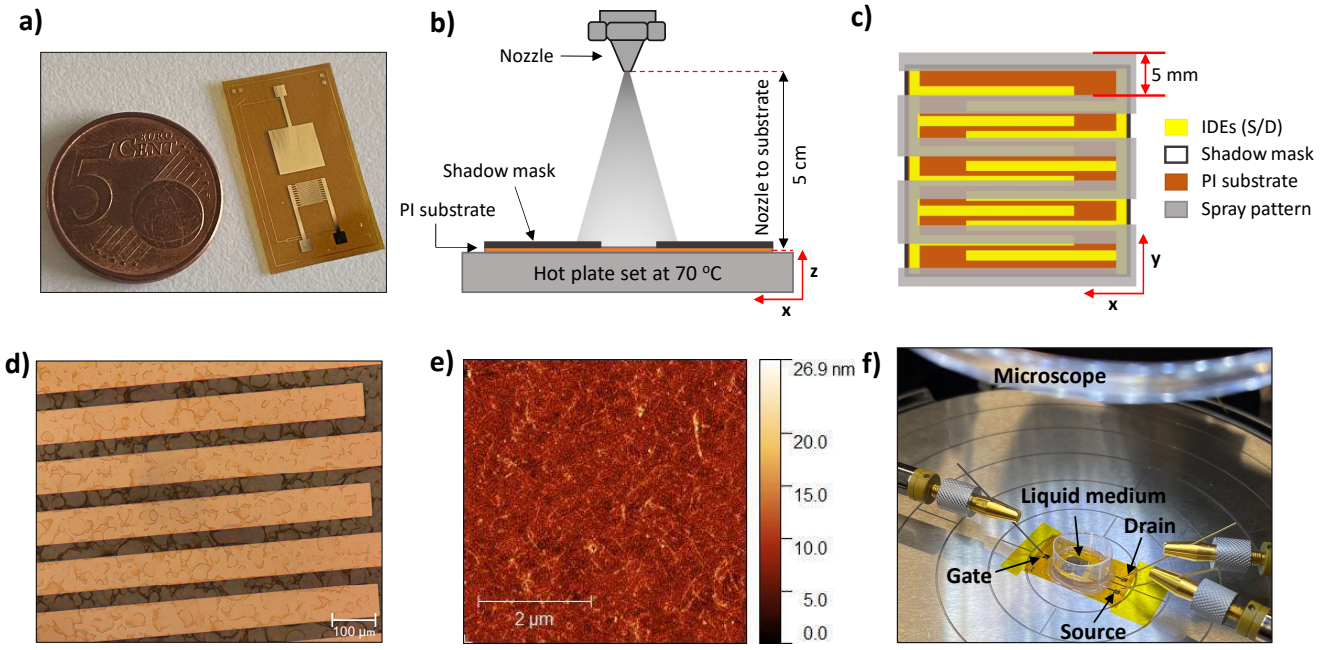

Fig. 1. a) Picture of gold electrodes (planar gate and interdigitated source and drain contacts) on flexible polyimide (PI) substrate. b) Scheme of the used spraying setup that is automated in x-y-z direction. c) Top view of the spraying pattern used for the deposition of the CNTs-CMC (sodium carboxymethyl cellulose) dispersions. The grey line represents the movement of the spraying valve during the deposition. d) Optical microscope picture of the as spray-deposited CNTs-CMC dispersion on gold interdigitated electrodes. d) Atomic force microscope (AFM) micrograph of the spray-deposited CNTs on the EG-FET, after the removal of the CMC matrix. f) Picture of the used electrical setup, with highlights of the different parts.

The semiconducting active area of the devices was patterned using a metallic shadow mask (8.9 mm^2) during the spray deposition. Figure S2 represents the spray coating setup used in this work. During the deposition process, the spraying valve described a serpentine shape above the active area of the device, to ensure homogeneous deposition of the material, as visualized in Figure 1c). A complete passage of the spraying valve is what we define as a ”layer” of CNTs and, as it will be thoroughly discussed in the rest of this work, the number of layers is one of the key parameters that needs optimization to achieve good performance of the EG-CNTFET. When using the spray deposition technique, three different deposition regimes can be achieved: wet, dry and intermediate [30]. Using the optimized parameters above mentioned, an intermediate deposition regime is achieved: as described in [31], using this regime the droplets reach the substrate when they are still in liquid form, merging with previously deposited material and quickly drying up to form an homogeneous thin film. An optical image of the as spray-deposited CMC-based SWCNT dispersion at the intermediate regime is presented in Figure 1d. Four different number of CNT layers, 18, 24, 30 and 36, were spray-deposited onto the IDEs (below 18 CNTs layers a proper electrical connection between the IDEs was not achieved). After spraying, to both remove the CMC and to increase the conductivity of the CNT network, the devices were immersed in 2.90 M nitric acid for 1h at room temperature [32], followed by a water bath for 10 min, and a final drying step on the hot plate at 100°C for 1h. The morphology of spray-deposited CNTs layers was evaluated by atomic force microscope (AFM) imaging (Nanosurf Core-AFM, Switzerland), operating in dynamic force mode. Figure 1e shows an AFM micrograph of the spray-deposited CNTs film (after acid and water bath treatment) for an EG-CNTFET fabricated on flexible polyimide (PI) substrate.

B. EG-CNTFET functionalization

To be able to use EG-CNTFETs as biosensing devices, a proper functionalization with a bio-recognition element is

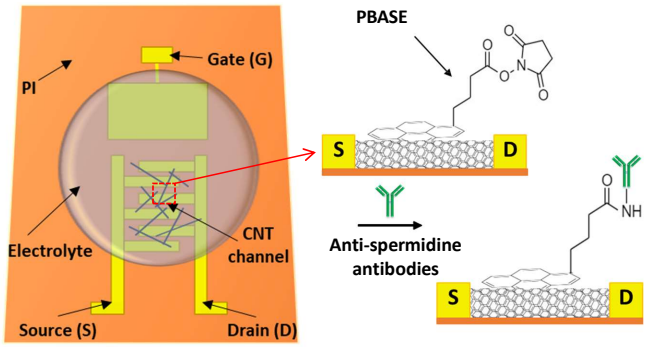

Fig. 2. Schematic top-view of a flexible planar electrolyte-gated field-effect transistor (EG-FET) based on semiconducting carbon nanotube (CNT) channel (EG-CNTFET). The zoom of bio-functionalized CNT channel with anti-spermidine antibodies using 1-Pyrenebutyric acid N-hydroxysuccinimide ester (PBASE) as a linker molecule.

required, in order to achieve a selective response toward a certain analyte. In this work, to achieve selective response for the analyte of interest (spermidine), the CNT channel was functionalized with anti-spermidine antibodies (as visualized in Figure 2). Antibodies are protein-based bio-recognition elements often use in EG-CNTFET-based biosensing due to specific chemical antibody-antigen interaction leading to selectivity toward certain analyte [17], [33]. The main drawback of CNTs, when used for biosensing, remains their hydrophobicity. As antibodies are hydrophilic molecules, the controlled immobilization of them onto hydrophobic nanotubes is extremely challenging. Challenging is also the bio-functionalization of nanotubes without damaging their electronic properties (the sp^2 hybridization must remain stable). In this regard, non-covalent functionalization strategies can be used. Here, a non-covalent functionalization step was performed, using 1-Pyrenebutyric acid N-hydroxysuccinimide ester (PBASE) as a binder molecule (see Figure 2). 50 μl of 5 mM PBASE were drop-casted on top of the semiconducting channel and allowed to interact with the nanotubes for 2h at room temperature [10], [12]. The benzene rings of PBASE attached to the CNTs due to the $\pi-\pi$ interactions, while the NHS-ester ligand remained free to react with primary amines (present in antibodies) yielding stable amide bonds. The PBASE was prepared in methanol by sonication in a water bath for at least 5 minutes before use. After functionalization, the PBASE excess was removed by rinsing the devices in pure methanol. Afterward, 10 μl of the polyclonal anti-spermidine antibodies were drop-casted onto the functionalized channel and incubated overnight at 4°C, to covalently bound to the NHS-ester ligands [34]. Furthermore, achieving a proper insulation of the CNT channel to reduce as much as possible the nonspecific binding is crucial for the successful realization of EG-CNTFET-based biosensors. To ensure specific binding, a blocking step was performed, 10 μl of bovine serum albumin (BSA) were drop-cast onto the devices and incubated at room temperature for 2h. After this blocking step, the current changes recorded by the transfer curve are attributed only to the interaction between the antis-permidine antibodies and the spermidine.

C. EG-CNTFET-based immunosensors characterization

The electrical characterization of the EG-FET devices was performed using a probe station, connected to a Keysight B1500A semiconductor device parameter analyzer (Figure 1f). The devices were tested at room temperature in ambient air conditions. Prior to the functionalization and the addition of the electrolyte, the source-drain resistance of the devices was measured, by sweeping the source-drain voltage V_{DS} from -0.5V to +0.5V and measuring the source-drain current I_{DS}. Three devices were tested for each number of layers. The effect of the channel resistance in the performance of the EG-CNTFET was investigated, by comparing four groups of devices fabricated with different number of spray-deposited CNT layers (18, 24, 30 and 36).

After functionalizing and adding the electrolyte (1 mM PBS) transfer and output characteristics were recorded. Two devices were tested for each number of layers. Prior to testing the EG-CNTFETs as biosensors for spermidine detection, to get insight into the stability of the electrical characteristics of the EG-CNTFETs after the functionalization protocol, the transfer characteristics were recorded until a stable I_{DS} was achieved in 1 mM PBS. The electrical response was recorded every 5 min for a total time of 20 min. The transfer characteristics were recorded sweeping the gate-source voltage V_{GS} from +0.8 V to -0.8 V, while maintaining the V_{DS} constant at -0.1 V. The output characteristics were recorded varying the V_{DS} from 0 V to -0.6 V for different values of V_{GS} (from +0.2 V to -0.8 V, with -0.2 V steps). The devices were then characterized in terms of threshold voltage (extracted from the I_{DS} in linear region), I_{ON}/I_{OFF} ratio and subthreshold swing SS.

The developed EG-CNTFET-based immunosensors were tested for the following spermidine concentrations: 10^{-3}, 10^{-2}, 10^{-1}, 10^{0}, 10^{1}, and 10^{2} nM. The spermidine concentrations were added successively, and after each addition, the system was left to stabilize for 5 min before recording both the transfer and the output curves, to allow the spermidine diffusion in the total volume of the PBS.

III. Results

A. EG-CNTFET operation

The main parameters extracted from the electrical characterization of the devices are summarized in Table I. The source-drain resistance values varied from around 1400 kΩ to circa 1.5 kΩ, depending on the number of layers of the spray-deposited CNTs (see Figure 3). The trend is consistent with the typical percolative behavior of CNT random networks [35]: increasing the number of CNT layers leads to a more dense network, i.e., to an overall increase in the channel conductivity.

The devices with 18 layers of spray-deposited semiconducting CNTs (source-drain resistance in average 1400 kΩ) did not show the behavior of an EG-FET (see Figure 4a), as the drain current was in the same range as the gate current:

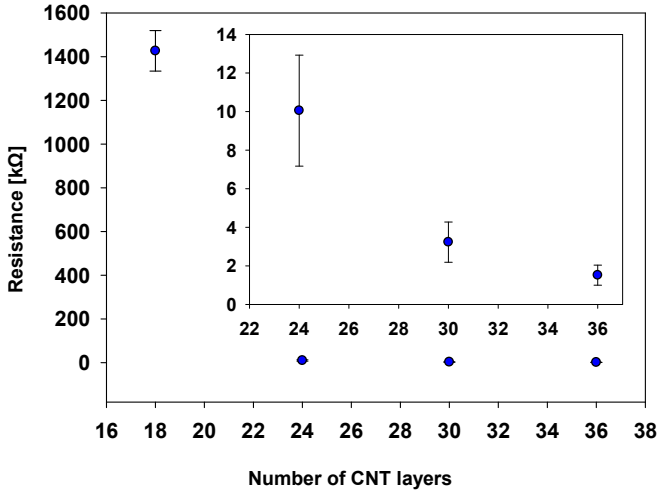

Fig. 3. Source-drain resistance measured for EG-CNTFETs fabricated depositing different number of CNT layers. Inset: the zoom of resistance values for 24, 30 an 36 layers of CNTs (the y-axis 0 to 14 kΩ).

TABLE I
EG-CNTFET BIOSENSORS PARAMETERS VS NUMBER OF SPRAY-DEPOSITED CNT LAYERS

CNT layers	**Source/Drain Resistance [kΩ]**	I_{ON}/I_{OFF} **[A/A]**	SS **[mV/decade]**	R^2	**Sensitivity [μA/decade]**
18	1426 ± 92.9	-	-	-	-
24	10.05 ± 5.29	214 ± 68	526.66 ± 126.57	0.738 ± 0.464	-1.03 ± 0.89
30	3.23 ± 0.85	496 ± 331	430.66 ± 96.52	0.955 ± 0.057	-1.14 ± 0.48
36	1.52 ± 0.42	365 ± 167	428.00 ± 48.66	0.951 ± 0.007	-2.45 ± 0.27

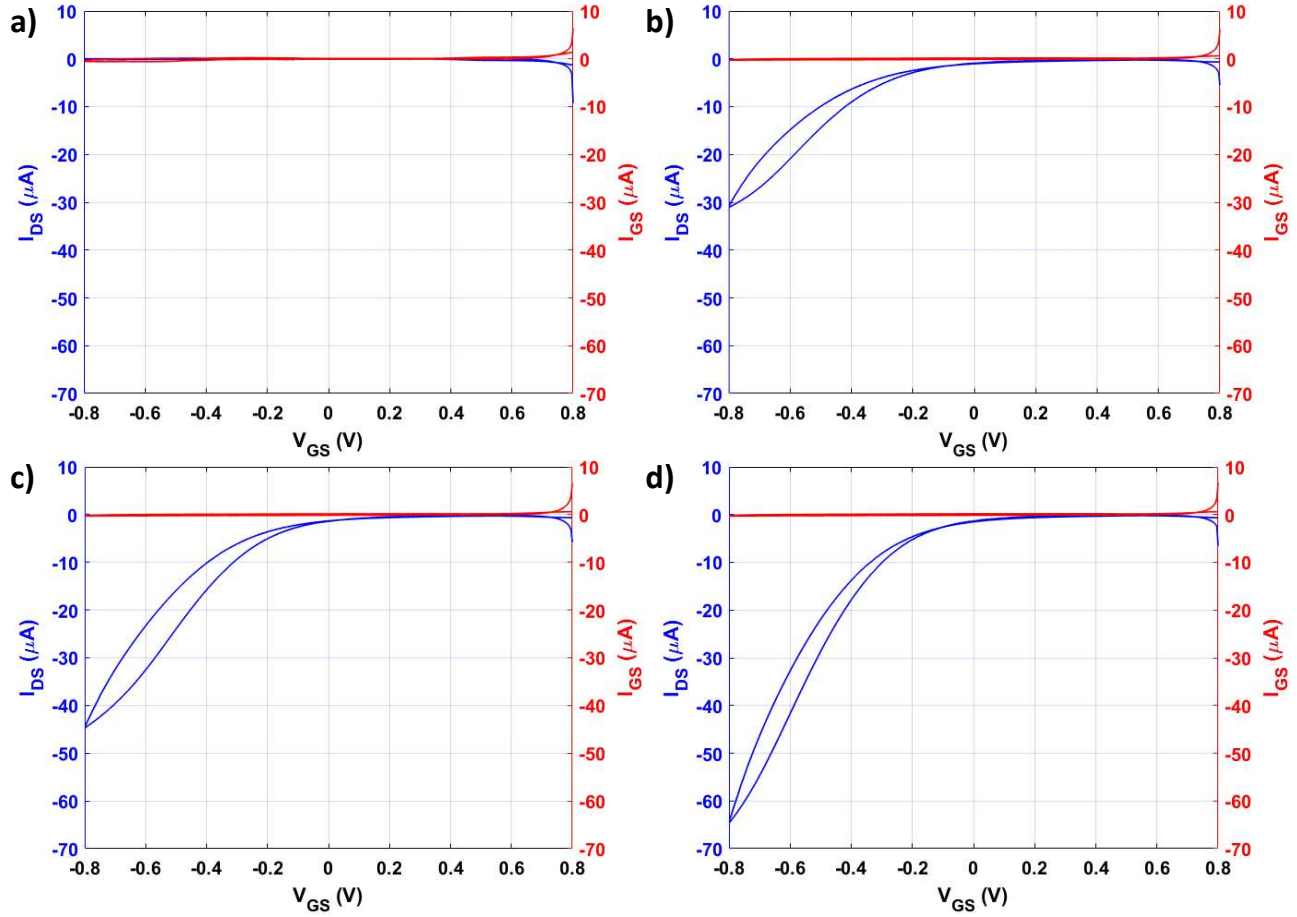

Fig. 4. Transfer characteristics for the fabricated EG-CNTFETs with different numbers of CNT layers: a) 18, b) 24, c) 30 and d) 36. Phosphate buffer saline (PBS) 1mM (120 µL) was used as the electrolyte.

our assumption is that when the conductivity of the CNT channel is too low, the formation of the EDL is not possible because not enough ions are attracted at the interface between the semiconducting channel and the electrolyte. The devices fabricated by increasing the number of CNT layers (i.e., increasing the conductivity of the channel, source-drain resistance of about ≤ 10 kΩ), showed typical EG-FET behavior when characterized in 1 mM PBS (see Figure 4b, 4c and 4d for the device fabricated using 24, 30 and 36 CNT layers, respectively). For all of these devices, the CNTs showed the expected p-type behavior and the gate-source current (I_{GS}) was (in absolute value) lower than the I_{DS}, during the entire sweep, by at least two orders of magnitude. The devices were characterized measuring the output characteristics as well, and the same p-type behavior was observed (as presented in Figure S3).

The devices with 24 layers of CNTs showed an average I_{ON}/I_{OFF} ratio of 214 A/A, in line with previous work of Joshi *et al.* [36], a threshold voltage of 0.4V and an average SS of 526 mV/decade. The devices with 30 and 36 layers of CNTs showed average I_{ON}/I_{OFF} ratio of 469 A/A and 365 A/A, respectively, and average SS of 431 and 428 mV/decade, respectively, while maintaining a threshold voltage of 0.4V. Hence, increasing the number of CNT layers from 24 to 30

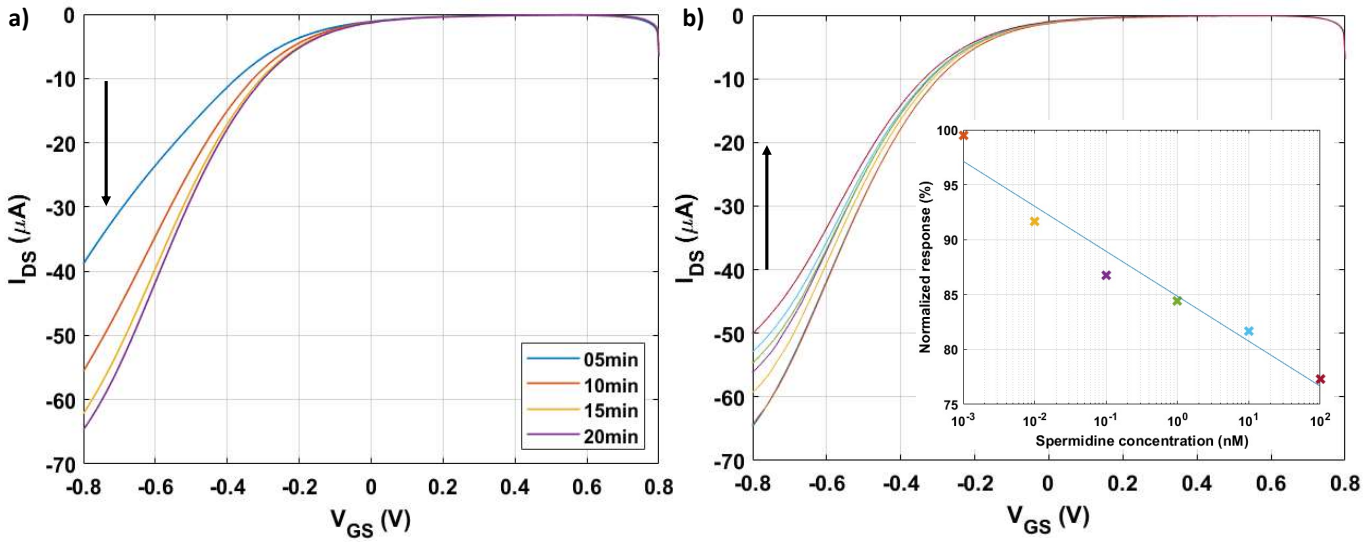

Fig. 5. a) Time dependence of drain-source current (I_{DS}) versus gate bias (V_{GS}) at a drain-source bias (V_{DS}) of -0.1 V for an EG-CNTFET fabricated with 36 layers of CNTs. b) Transfer characteristics of the same device, after the functionalization with anti-spermidine antibodies, for different concentrations of spermidine: the presence of the analyte causes a decrease (in absolute value) in the measured current. Inset: concentration of spermidine vs. normalized response, defined as the drain-source current at V_{GS} = -0.8V.

enhanced the I_{ON}/I_{OFF} ratio, by further increasing the CNT layers to 36 the I_{ON}/I_{OFF} ratio was reduced. Here we see a percolative behavior also for the off current: even if the on current always increases, for $>$ 36 CNT layers the increase in the off current is counterbalancing it, leading to an overall lower I_{ON}/I_{OFF}. The SS was much higher for the devices with 24 layers, while it was comparable for the devices with 30 and 36 layers, with the latter showing lower standard deviation. Figure S4 in the supporting document represents the log(I_{DS}) vs. V_{GS} for the fabricated EG-CNTFETs.

As for the stability in 1 mM PBS, an example of the recorded behavior is shown in Figure 5a: an increase in the absolute value of the I_{DS} was observed. Considering as reference point the absolute value of I_{DS} at V_{GS} = -0.8V, the device shown went from an initial value of -38.84 μA to -55.55 μA after 10 minutes of exposure to the PBS. After 20 minutes, the value stabilized at around 64.60 μA, with a difference between the last two points recorded of less than 4%. As visible in Table I, the variability in the electrical characteristics of the fabricated EG-CNTFET is considerable. This can be related to both the wide tube-to-tube dimension variation of the CNTs used and the low reproducibility of the spray technique, which led to high inter-sample variability. In fact, the high variability of the CNT-based FETs is well reported in the literature and remains challenging for their practical use [37]–[39].

B. EG-CNTFET-based immunosensors

The sensing mechanism of EG-CNTFET-based biosensors using antibodies as bio-recognition element is based on the immunocomplex formation between the target antigens with the antibodies immobilized on the CNT channel, triggering a change in the surface charges and therefore a measurable change of the I_{DS} [11]. As seen in Figure 5b, with increasing concentration of spermidine, a decrease in the absolute value of I_{DS} was observed due to the formation of the immunocomplex. These results are in agreement with the work presented by Belkhamssa *et al.* [33], where the authors have seen a decrease in drain current with the addition of the analyte to the immunosensor. Since spermidine is a positively charged molecule at physiological solution [40], [41], its attachment to the anti-spermidine antibodies caused a decrease to the absolute value of the I_{DS} due to electrostatic interaction between positively charged analytes and CNTs (i.e., the positive charge potential of the spermidine caused a depletion of holes in the semiconducting channel, hence less current).

For the EG-CNTFETs that we characterized also as biosensors (i.e., the EG-CNTFETs fabricated with 24,30 and 36 CNT layers), we recorded a linear response between the normalized current response (defined as the value of absolute value of the I_{DS} at V_{GS} = -0.8V, normalized to the same point of the I_{DS} recorded before starting adding the spermidine concentrations) and the logarithmic concentration of spermidine across the measured range of concentrations (the plots for stabilization in 1 mM PBS and the calibration curves for the devices with 24 and 30 CNT layers can be found in Figure S5 and S6). The coefficient of determination together with the sensitivities are presented in the Table I. Even if the devices with 30 layer of CNTs showed the best behavior in terms of I_{ON}/I_{OFF} ratio, the sensitivity of the devices is increased with the increased number of layers with -1.03 μA/decade, -1.14 μA/decade and -2.45 μA/decade for 24, 30 and 36 layers of spray-deposited CNTs, respectively. The increased sensitivity for denser CNT networks from the biosensing point of view is expected. Having denser CNT network results in fact in an increased surface area of the biosensor, hence in more binding sites available for the immobilization of the anti-spermidine antibodies, leading thereby to improved sensitivity. The limit of detection for all three devices is 0.01 nM of spermidine, being the lowest concentration that could be distinguished

from the signal obtained with 1 mM PBS.

IV. Conclusion

In this work, we present the manufacturing and characterization of EG-FETs based on spray-deposited semiconducting CNTs, on a flexible PI substrate. To understand the critical aspects of the semiconducting CNT channel and its direct correlation to the EG-FET device performance, we have investigated four different number of layers of CNTs (resulting in different source-drain resistances). Results clearly show that a percolation threshold for the semiconducting CNT channel must be reached in order to obtain the electron pathways needed to form the EDL, hence to trigger the EG-FET behavior. From our data, we can conclude that a CNT source-drain resistance of circa ≤ 10 kΩ should be reached to obtain an EG-CNTFET behavior. Furthermore, as a proof of concept, we demonstrated the detection of the polyamine spermidine with EG-CNTFETs functionalized with anti-spermidine antibodies. The tested devices (with 24, 30 and 36 CNT spray-deposited layers) showed linear responses between the normalized current response and the logarithmic concentration of spermidine across the measured range of concentrations (from 10^{-3} to 10^{2} nM), with increased sensitivity in function of increasing number of layers. To the best of our knowledge, there are no other reported works focusing on the optimization of the spray-deposited semiconducting CNTs for EG-CNTFET-based biosensors applications. The results obtained are promising for these devices to be used as a label-free biosensor for the quantification of polyamines. More research is needed to overcome the considerable variability of CNT-based EG-FET, with a possible solution including precise control of the diameter distribution of CNTs, use of highly pure semiconducting CNT (e.g., by selective growth), complete passivation of CNTs (in this case gate bio-functionalization must take place). However, the additional cost related to these additional/alternative processes in the device fabrication will need to be kept as low as possible, leading necessarily to reasonable trade-offs between device performance and cost.

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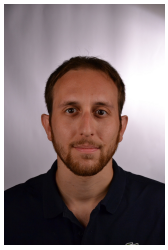
Bajramshahe Shkodra obtained the M.Sc degree in the field of Analytical and Environmental Chemistry from the University of Prishtina, Kosovo (2017). Currently, she is a PhD student at the Free University of Bozen-Bolzano, working on the development of flexible and printed biosensors at the Sensing Technologies Lab. Her main research area includes the design, fabrication, and characterization of biosensors for food application.



Mattia Petrelli received the M.Sc. degree in electronic engineering from the University of Salerno, Italy, in 2017. He is currently a Ph.D. student in advanced-systems engineering at the Free University of Bozen-Bolzano, Italy. His project is aimed at the design, fabrication and characterization of real-time wearable solutions to monitor the muscles' activity, to provide immediate feedback to the athlete about their physiological status.

Martina Aurora Costa Angeli received her master's degree in biomedical engineering (2016) from Politecnico di Milano (Italy), where she obtained the Ph.D. in materials engineering in 2020 working on the development of different types of deformable printed low-cost sensors for strain measurement. Currently, she is a post-doctoral researcher at the Sensing Technologies Lab of Free University of Bolzano (Italy). Her current research work is focusing on the design of low-cost flexible sensors for environmental and human monitoring, and food safety.

AKM Sarwar Inam is currently working in the Ph.D. program at the Sensing Technologies Lab of Free University of Bozen-Bolzano, Bolzano, Italy. He is doing research work on the realization of electrochemical sensors for the food safety assessment. He was working as an Assistant Professor in the Department of Nutrition and Food Engineering, Daffodil International University, Bangladesh. Previously he completed his Masters in Food Science, Technology and Nutrition from KU Leuven in Belgium (2012-2014) with Erasmus Mundus scholarship and Bachelor in Food Engineering in Bangladesh Agricultural University, Bangladesh (2007).

Paolo Lugli graduated in physics from the University of Modena and Reggio Emilia, Italy, in 1979. He received the M.Sc. and Ph.D. degrees in electrical engineering from Colorado State University, Fort Collins, CO, USA, in 1982 and 1985, respectively. He is currently the Rector of the Free University of Bozen-Bolzano. He has authored more than 350 scientific articles.

Luisa Petti received the M.Sc. degree in electronic engineering from the Politecnico di Milano, Milan, Italy, in 2011, and the Ph.D. degree in electrical engineering and information technology from ETH Zürich, Zürich, Switzerland, in 2016. She is currently an Associate Professor with the Free University of Bozen-Bolzano, Bolzano, Italy, where she researches on flexible and printed electronics for a wide range of applications, including food engineering and biotechnology.