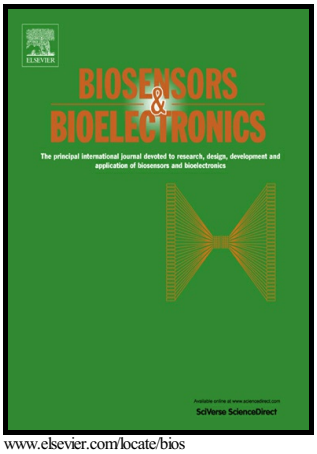

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PII: S0956-5663(18)30402-0
DOI: https://doi.org/10.1016/j.bios.2018.05.048
Reference: BIOS10506

To appear in: *Biosensors and Bioelectronic*

Received date: 22 March 2018
Revised date: 16 May 2018
Accepted date: 27 May 2018

Cite this article as: Shimaa Eissa, Nawal Alshehri, Mai Abduljabbar, Anas M. Abdel Rahman, Majed Dasouki, Imran Y. Nizami, Mohammad A. Al-Muhaizea and Mohammed Zourob, Carbon nanofiber-based multiplexed immunosensor for the detection of Survival Motor Neuron 1, Cystic Fibrosis Transmembrane Conductance Regulator and Duchenne Muscular Dystrophy proteins, *Biosensors and Bioelectronic,* https://doi.org/10.1016/j.bios.2018.05.048

Carbon nanofiber-based multiplexed immunosensor for the detection of Survival Motor Neuron 1, Cystic Fibrosis Transmembrane Conductance Regulator and Duchenne Muscular Dystrophy proteins

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Abstract

Simultaneous and point-of-care detection of multiple protein biomarkers has significant impact on patient care. Spinal Muscular Atrophy (SMA), Cystic Fibrosis (CF) and Duchenne Muscular Dystrophy (DMD) are well known progressive hereditary disorders associated with increased morbidity as well as mortality. Therefore, rapid detection of biomarkers specific for these three disorders in newborns offers new opportunities for early diagnosis, delaying symptoms and effective treatment. Here, we report the development of a disposable carbon nanofiber (CNF)-based electrochemical immunosensor for simultaneous detection of survival motor neuron 1 (SMN1), cystic fibrosis transmembrane conductance regulator (CFTR) and DMD proteins. The CNF-modified array electrodes were first functionalized by electroreduction of carboxyphenyl diazonium salt. Then, the immunosensor was fabricated by the covalent immobilization of the three antibodies on the working electrodes of the array sensor via carbodiimide (EDC/NHS) chemistry. Simultaneous detection of CFTR and DMD and SMN1 was achieved with high sensitivity and detection limits of 0.9 pg/ml, 0.7 pg/ml and 0.74 pg/ml, respectively. The multiplexed immunosensor has also shown strong selectivity against non-specific proteins. Moreover, high recovery percentage was obtained when the immunosensor was applied in spiked whole blood samples. This voltammetric immunosensor offers cost effective, easy to use, rapid and high throughput potential screening method for these three hereditary disorders using only few drops of blood.

Keywords: biosensor, Cystic Fibrosis (CF), Duchenne Muscular Dystrophy (DMD), Dystrophin, Spinal Muscular Atrophy (SMA), square wave voltammetry, Survival Motor Neuron 1 (SMN1), voltammetry

1. Introduction

In some genetic conditions, early screening and diagnosis plays a major role in taking successful therapeutic decisions for disease management and it can be achieved via the detection of "DNA, RNA, protein, or analyte" related disease-specific biomarkers (Radha Shanmugam et al. 2017) in the patient's representative biological matrices. The differential expression of the disease biomarker indicates irregularities in cell function, pathological responses or results from various therapeutic interventions (Neumann 2015).

Spinal Muscular Atrophy (SMA), Cystic Fibrosis (CF) and Duchenne Muscular Dystrophy (DMD) are well characterized progressive monogenic hereditary diseases that cause significant health problems leading to high mortality. SMA is caused by homozygous mutations (deletions) in the survival motor neuron 1 (*SMN1*) gene (Farrar and Kiernan 2015). This mutation usually results in very low or absent expression of the SMN protein in the cells and tissues of SMA patients (Farrar and Kiernan 2015). SMA is a progressive neuromuscular disorder that leads to muscle weakness and may result in death due to respiratory failure.

Cystic fibrosis is another autosomal recessive disease (Peters et al. 2011) which leads to dysfunctional chloride transport across the epithelium of the respiratory, gastrointestinal and genitourinary tracts resulting in damage of those organs. Cystic fibrosis results from mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. Recurrent and frequent respiratory infections, bronchiectasis and lung failure can cause significant disability and even early death (Peters et al. 2011) while blockage of the spermatic cord in male patients leads to infertility (Marcorelles et al. 2012).

Duchenne (limb girdle) muscular dystrophy is also a common X-linked genetic disorder characterized by progressive muscle degeneration and is caused by absence or markedly reduced expression of the dystrophin (DMD) protein (Bushby et al. 2010). Muscle weakness appears as early as 3 years of age affecting hips, pelvic area, shoulders and thighs and then arms and legs. Later it can affect respiratory (intercostal) and cardiac muscle leading to potentially life threatening respiratory failure and dilated cardiomyopathy, respectively.

Because of the serious complications that can result from CF, DMD and SMA disorders and the recent advent of novel therapies, there is growing interest in the early screening and diagnosis for these disorders in newborn babies (Sander et al. 2000). Over the past few years, several analytical techniques have been developed for the diagnosis of these disorders through the detection of their proteins. Mass spectrometry has been utilized for the detection of CFTR (Baldwin 2004; Cravatt et al. 2007; Goldstein et al. 2007; McClure et al. 2011), SMN (Fuller et al. 2015; MacKenzie 2010; Wu et al. 2011) and DMD (Anthony et al. 2014). High-performance liquid chromatography for SMN detection was also used (Kao et al. 2006). Real-time (Maksimovic et al. 2012) and multiplexed (Kawamura 1997) PCR for DMD, CFTR and SMN (Liu et al. 2016) are also routinely used. However, these methods are still expensive and require relatively bulky and expensive equipment to run the assays on. Various quantitative immunoassays for these three proteins such as western blot (WB) for SMN (Coovert et al. 1997; Hsieh-Li et al. 2000; Sangiuolo et al. 2005; Wu et al. 2011), CFTR (Farinha et al. 2004; Goldstein et al. 2007) and DMD (Ferrer et al. 2000; Kawamura 1997) have also been reported. Moreover, SMN, CFTR and DMD have been detected using immunoprecipitation (Farinha et al. 2004), immunocytochemical methods (Davidson et al. 2009), immunofluorescence (Pellizzoni et al. 2001; Peters et al. 2011; Rossoll and Bassell 2009), immunohistochemistry (Chang et al.

2001; Martinez et al. 2012; Monani et al. 2000), immunostaining (Ferrer et al. 2000) and electrochemiluminescence immunoassays (Steinkellner et al. 2015; Zaworski et al. 2016). High sensitivity enzyme-linked immunosorbent assays (ELISA) have also been reported for SMN (Kobayashi et al. 2011; thi Man et al. 2008), CFTR (Liu et al. 2014; Peters et al. 2011) and DMD (Anthony et al. 2014; Ferrer et al. 2000). However, immunoassays particularly ELISA need several reagents, involve multiple steps and are not suitable for point-of-care testing or high throughput screening (Lisdat and Schäfer 2008).

Recently, few biosensors based detection for CFTR, DMD and SMN have become attractive alternatives to the conventional detection assays. Optical transducers have been used for the detection of SMN1 gene defects (Piunno 2009). Furthermore, surface plasmon resonance (SPR) has been employed for the detection of SMN (Watterson et al. 2004) as well as CFTR proteins (Feriotto et al. 2001; Trouve et al. 2015). However, optical biosensors are expensive and still do not satisfy current needs. Therefore, there is a strong clinical need for a simple, inexpensive, sensitive and specific method which offers high throughput simultaneous and multiplexed detection of these three biomarkers in a single human blood sample.

Different transduction strategies have been used for multiplex analyte detection. Electrochemical biosensor-based detection is one of the most important ways that can be widely implemented in a multiplexed label –free format for the simultaneous detection of multiple analytes. In these devices, the captured protein biomarker on the electrode surface is converted to a measurable electrical signal.

The properties of the electrode material play a major role in determining the sensitivity, selectivity and stability of the biosensor. Nanostructures-modified electrodes thus, offer great

opportunities for developing robust biosensor platforms due to their large surface area, high conductivity and enhanced electrochemical response (Zhang et al. 2009).

Carbon nanaofibers (CNF) are cylindrical nanostructures characterized by different stacking arrangements of graphene sheets (Huang et al. 2010; Komori et al. 2016; Evtugyn et al. 2014). CNFs have the same mechanical strength and electric properties as carbon nanotubes (CNTs) (Vamvakaki et al. 2006). However, CNFs have more controllable length and diameter than CNTs. Moreover, the outer surface of CNFs contains more defective sites than CNTs (Huang et al. 2010). These defects increase the electron transfer efficiency of electroactive species on the CNFs electrode surface (Banks and Compton 2005). Another unique advantage of CNF is that all the surface of the CNFs can be functionalized without degradation of the carbon backbone structure offering a larger surface area for immobilization of biomolecules.

Electrode functionalization is one of the most crucial steps in the fabrication of biosensors in order to ensure effective immobilization of biomolecules. Among the common surface modification methods, electrochemical reduction of diazonium cations appeared to be very effective and capable of addressing the molecules to microarray electrodes (Belanger and Pinson 2011; Randriamahazaka and Ghilane 2016). This method is also easy, fast (takes few seconds) and can be used to attach a variety of functional groups to the surfaces in a controllable manner leading to the highly stable functionalized surface. We have recently compared the diazonium grafting on different carbon nanostructure-modified electrodes and their analytical performance in immunosensors (Eissa et al. 2018). It was concluded from this study that the CNF-modified electrode was the most responsive sensor for protein detection.

Recently, electrochemical immunosensors for SMN1 (using voltammetric measurements) (Eissa et al. 2018), as well as CFTR and DMD (using impedance measurements) (Alshehri et al.

2017) have been reported. However, multiplexed immunosensor for the three analytes would enable faster and lower cost detection way using a small blood sample volume. In this work, we demonstrate the development of a multiplexed immunosensor for CFTR, DMD and CFTR using CNF-modified array disposable electrodes that can potentially be used in screening and point-of-care testing.

2. Experimental

2.1. Materials and Reagents

All the materials, reagents and equipments used in this work are described in details in the supporting information file.

2.2. Procedures

2.2.1. Preparation of carbon nanofiber- modified electrodes

The CNF modified DEP electrodes were prepared by drop casting. Two microliters (2 µL) of CNF solution (1 mg/ml in DMF) were placed onto each electrode surface from the eight working electrodes and allowed to dry at room temperature overnight. The excess CNF particles were then removed from the surface by gentle rinsing with milli-Q water.

2.2.2. Cyclic voltammetry functionalization of the carbon nanofiber-modified array electrodes and fabrication of the multiplexed immunosensor

The immunosensor was fabricated on the CNF-modified electrode array (scheme 1A). The Fabrication steps are explained in details in the supporting information file.

2.2.3. Square wave voltammetry detection measurements

For the detection measurements of SMN1, CFTR and DMD proteins, SWV were recorded in 5 mM $[Fe(CN)_6]^{3-/4-}$ redox solution prepared in 10 mM PBS, pH 7.4. The parameters of the SWV were as follows: the potential range: 0.3 to -0.1 V, scan rate: 125 mV/s, frequency: 25 Hz,

amplitude: 20 mV, interval time: 0.04 s and step potential: -5 mV. Base-line corrections were applied for all the SWV data.

The immunosensing experiments were done by incubating the immunosensor array chip with the specific proteins at different concentrations for 45 min. The electrodes were then washed with PBS buffer, pH 7.4 and subjected to SWV measurements as described above. For the selectivity experiments, the array electrodes were incubated with 1 ng/ml of non-specific proteins for 45 min. After washing, the chips were measured using SWV.

The immunosensor response was calculated as the percentage change in the SWV reduction peak current $(i-i^o)/i^o$ % of the $[Fe(CN)_6]^{3-/4-}$ redox couple for each protein. i^o is the SWV peak current before binding for each electrode on the array and i is the peak current measured after binding with the different concentrations of the specific or non-specific proteins.

2.2.4. Testing the multiplexed immunosensor in whole blood samples

Patient samples collection

Patients with confirmed genetic defects for CFTR, DMD and SMN were recruited in this study. The blood samples were collected from subjects who attended the cystic fibrosis and pediatric neurology clinics at King Faisal Specialist Hospital & Research Center (KFSHRC). All patients signed an informed consent form by the declaration of the ethics committee and answered a baseline questionnaire regarding the symptoms of their diseases. The institutional review board (IRB) at KFSHRC, (RAC No. 2160 031, and RAC No. 2170 001) approved all research involving patient materials. The protocols for testing the spiked whole blood, Dried Blood Spots (DBS) sample elution as well as the ELISA are described in detail in the supporting information file.

3. Results and discussion

3.1.Scanning electron microscopy imaging of the carbon nanofibers-modified array electrodes

In this work, we used commercially synthesized CNF material produced via chemical vapour deposition process. According to the manufacturer, the nanofibers present helicoidal structure with average diameters ranging from 40 to 80 nm and average length varying from 0.5 to 1.5 µm. Transmission Electron Microscopy (TEM) image of a single nanofiber is shown in Fig. 1A. Scanning electron microscopy (SEM) has been also used to characterize the morphology of the array screen printed electrode surface before and after modification with CNF. As shown in Fig. 1B and C, significant change was observed in the morphological structure of the array electrode surface after CNF deposition. The original graphite carbon electrode surface exhibited a typical multilayer structure (Fig. 1B). However, the CNF-modified electrodes showed a well-packed CNF layer in which some rods can be distinguished. Therefore, it is obvious that the CNF-modified surface provided larger surface area than the original carbon electrodes.

3.2.Cyclic voltammetry grafting of the carboxyphenyl groups on the carbon nanofibers-modified electrodes

The electrografting of the carboxyphenyl layer on the CNF electrodes was done by the electroreduction of the prepared diazonium salt using CV sweeping from 0.4 and -0.6 V. Figure 2A shows three consecutive CV scans of CNF electrode in the diazonium salt solution. As expected the typical single irreversible cathodic peak for the diazonium reduction was seen in the first CV cycle at -0.25 V. This peak corresponds to a one electron transfer process in which nitrogen molecule is eliminated from the diazonium salt leading to the formation of an aryl radical. Then, the radical reacts with the CNF surface forming covalent bond as previously

reported elsewhere (Belanger and Pinson 2011). In the second CV cycle, the reduction peak intensity was decreased likely due to the attachement of carboxyphenyl groups to the surface that lead to partial retardation of the electron transfer. However, in the third CV cycle, further decrease in the peak current was obtained. This indicates the full coverage of the CNF surface with the aromatic film. The gradual passivation of the CNF electrode with multiple CV cycles was in agreement with our previously published work on different carbon nanomaterials (Eissa et al. 2018; Eissa et al. 2013; Eissa et al. 2012). It is worth noting that multilayers of carboxyphenyl group can be formed with multiple CV scans which can negatively impact the biosensor performance (Eissa et al. 2018; Eissa et al. 2013; Eissa et al. 2012).

3.3.Characterization of the functionalized CNF electrodes using Raman spectra and X-ray photoelectron spectroscopy

The CNF electrodes were characterized using Raman spectroscopy and XPS before and after electrografting. Fig. 2B shows the Raman spectra of the CNF surface before functionalization exhibiting the characteristic Raman signature of the carbon nanomaterials. Two prominent peaks were observed; a D peak and a G peak which correspond to the sp^2-hybridised carbon vibrations and sp^3- like defects in the structure, respectively. After the carboxyphenyl grafting with 3CV scans, an enhancement of the D peak intensity was observed indicating the increase in the defect density on the CNF surface. It is widely established that the ratio between the D and G band intensities (D/G ratio) gives an estimation of the degree of disorder in the carbon structure. Thus, the increase of the I_D/I_G ratio from 0.41 before functionalization to 0.82 after electrografting confirms the formation of the covalent bond between the carboxyphenyl groups and the CNF lattice leading to breaking of the translational symmetry of the C–C sp^2 bonds on the CNF surface and the formation of localized C–C sp^3 bonds.

The electrografting was also investigated using XPS. As shown in Fig. S1, two characteristic peaks were observed in the XPS survey spectra of the CNF electrode before functionalization. The peaks were seen at 284 and 532 eV for the carbon 1s and oxygen 1s, respectively. After electrografting, the oxygen peak was significantly enhanced indicating the formation of the carboxylic layer on the CNF surface. Fig. 2C and D show the XPS C1s high resolution spectra of the CNF before and after grafting. A peak at 288.8 eV appeared after grafting which is characteristic for the carboxylic acid groups.

3.4.Characterization of the multiplexed immunosensor fabrication steps on the CNF array electrodes

In order to characterize the stepwise fabrication of the CFTR, SMN1 and DMD immunosensor, SWV was recorded after each step in ferro/ferricyanide redox couple. As shown in Fig. 3, the CNF electrodes showed a well-defined cathodic peak for the reduction of the redox molecules. After electrografting using 3 CV scans, the ferro/ferricyanide reduction peak dramatically dropped. This is attributed to the blocking effect of the carboxyphenyl groups which retards the electron transfer as well as the negative charge of the carboxylic groups that repels the negatively charged redox molecules. However, after EDC/NHS activation and the attachment of the different antibodies, the SWV reduction peak current increased likely due to the positive charge of the antibodies which could neutralize the carboxylic groups (Eissa et al. 2018; Eissa et al. 2013). Finally, the binding of the SMN1, DMD, and CFTR proteins to the immunosensor surface led to further enhancement of the peak current in the three cases. The current increase in the case of CFTR and SMN immunosensors could be attributed to the positive charge of these proteins at pH 7.4 (isoelectric points around 8.0) (Battaglia et al. 1997).The positive charge of the proteins

might lead to attraction of the ferro/ferricyanide redox anion to the electrode surface and thus, raising the reduction current. On the other hand, in the case of the DMD electrodes, the increase in the signal might be due to the conformational change of the antibody upon binding with the DMD antigen that led to better access of the redox molecules to the CNF surface thereby increasing the current.

3.5.Dose response of the carbon nanofiber-based multiplexed immunosensor

In order to investigate the analytical range of the array immunosensor for the three proteins, the immunosensor was incubated with different concentrations of SMN1, CFTR and DMD solutions in PBS buffer for 45 min. After washing, the array immunosensor was measured in ferro/ferricyanide redox solution by SWV. The immunosensor response was determined as the percentage change in the SWV reduction in peak current before and after binding with the respective protein. Fig. 4 shows the gradual enhancement of the SWV signals of SMN1 (Fig. 4A), CFTR (B) and DMD (C) electrodes after binding with different concentrations of these proteins (from 1 pg/ml to 1 µg/ml). The calibration curves of the immunosensor are shown in Fig. 4D, E and F. A linear response was obtained in the case of SMN1 (D) and CFTR (E) electrodes within a wide range from 1 pg/ml to 1 µg/ml. However, for DMD (F), the linear range was from 1 pg/ml to 10 ng/ml after which no further increase in current was obtained. This is likely due to saturation of the immunosensor surface with the maximum number of DMD molecules. The linear regression equation was: $(i - i^o)/i^o\% = 133.96 + 32.3 \log C$ [ng/mL], R = 0.989 for CFTR, $(i - i^o)/i^o\% = 283.08 + 74.9 \log C$ [ng/mL], R = 0.981 for SMN1 and $(i - i^o)/i^o\% = 289.2 + 66.7 \log C$ [ng/mL], R = 0.979 for DMD. The multiplexed immunosensor showed a low detection limit of 0.9, 0.7 and 0.74 pg/ml for CFTR, DMD and SMN1, respectively. The

limits of detection (LOD) were calculated based on 3 σ/b, where σ is the standard deviation of the immunosensor after incubation in buffer and b is the slope of the straight line of the calibration curve. It is worth noting that the obtained LODs for our multiplexed immunosensor are lower than that reported with other methods such as ELISA and other immunosensors (Alshehri et al. 2017; Eissa et al. 2018; Masson et al. 2004; Zaworski et al. 2016) which indicates the excellent sensitivity of the immunosensor.

3.6.Selectivity of the CNF-based multiplexed immunosensor

It was extremely important to study the selectivity of the multiplexed immunosensor to their respective proteins in order to confirm that the response of each electrode is due to the specific binding. Each immunosensor as well as the control sensors were incubated with the three proteins (CFTR, DMD and SMN1) to investigate their cross reactivity. Fig. 5A shows the immunosensor response to the three proteins. It can be clearly seen that the sensor response for each protein to its respective sensor was much higher than the other two proteins. This insignificant response of the non-specific proteins was comparable with the response of the control sensor with the three proteins indicating high selectivity of the multiplexed immunosensor. Moreover, we also incubated the electrodes with BSA protein since the albumin is the most abundant protein in the blood and we have not seen any significant response indicating that the sensor can be applied in the blood without interference from the other proteins.

Fig. 5B shows the response of the sensor for the simultaneous detection of the three analytes. Upon the addition of DMD, the signal increase was seen only in the DMD electrodes, while weak signal change was seen in the CFTR and SMN1 electrodes. When a mixture of CFTR and SMN1 was incubated on the immunosensor, signal enhancement for both CFTR and

SMN1 electrodes was observed. However, increase in the signals for all electrodes was observed when a mixture of the three analytes was incubated on the sensor. These results confirm the feasibility of applying our immunosensor for multiplexed detection of CFTR, SMN1 and DMD proteins.

3.7. Stability and reproducibility of the multiplexed CNF array immunosensor

The multiplexed immunosensor stability was investigated after storage at 4 ^{o}C for two weeks in PBS buffer pH 7.4. The immunosensor was incubated in 1 ng/ml of CFTR, SMN1 and DMD. Then, the sensor response was recorded after washing. Non significant change in the signal response (2 %) was observed after a month indicating good stability of the CNF-based immunosensor. We attribute this stability to the stability of the used carbon nanomaterial as well the strong covalent attachment of the antibodies.

The reproducibility of the multiplexed immunosensor was also investigated by performing triplicate experiments for each analyte. The relative standard deviations of the measurements ranged from 4.0-7.0% indicating very good reproducibility of the sensor.

3.8.Application of the CNF-based multiplexed immunosensor in spiked human whole blood

With the aim of investigating the possibility of applying the multiplexed immunosensor in a real biological sample, the immunosensor was utilized to detect CFTR, SMN1 and DMD in human whole blood from a healthy volunteers and patients with confirmed genetic defects for CFTR, DMD and SMN. For the recovery experiment, the sample was lysed, spiked with 1 ng/ml of each protein then applied to the immunosensor surface. After incubation and washing, the immunosensor response was measured. Table S1 shows the high recovery percentage obtained in each case. For the DBS samples, after elution, the samples were subjected to analysis with both ELISA and the immunosensor. As shown in Fig. 5C, D and E, the level of the three proteins in

patient samples were significantly less than the control samples. These results were in good a agreement with the results obtained from ELISA indicating the potential utilization of the current assay for screening SMN1, CFTR and DMD in whole blood and its capability to distinguish the patient from the healthy samples.

Conclusions

In conclusion, a rapid, sensitive and low cost electrochemical immunosensor array was developed for the simultaneous detection of three clinically relevant proteins which are associated with hereditary disorders; SMA, CF and DMD. Carbon nanofiber-modified electrodes were used as a transducer for the immunosensor. The carbon nanofiber electrodes were functionalized via the electrochemical reduction of carboxyphenyl diazonium salt using cyclic voltammetry. The functionalized electrode arrays were then used for the antibodies immobilization and immunosensor fabrication. Based on the unique proprieties of CNF and the strength of the functionalized surface using diazonium reduction, a label-free detection scheme was utilized. Simultaneous detection of the SMN1, CFTR and DMD was achieved in a homogeneous solution with high degree of selectivity, thus demonstrating the potential utilization of our platform in the rapid high-throughput screening of multiple proteins. Low detection limits of 0.9, 0.7 and 0.74 pg/ml for CFTR, DMD and SMN1, respectively, were obtained indicating the high sensitivity of the platform. Moreover, the multiplexed immunosensor array was successfully applied in human whole blood samples.

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Fig. 1. Transmission Electron Microscopy image (DropSens, Asturias, Spain) of the CNF rod (A), SEM images of carbon screen printed electrodes surface (B) and CNF-modified electrodes (C). Scanning electron microscopy measurements were performed using an acceleration voltage of 3 kV, magnification= 50,074X and a working distance of 2 mm. ELISA plate reader (ELx800 BioTek) from BioTek (Winooski, USA)

Fig. 2. Successive cyclic voltammetry cycles for the electroreduction of 4-carboxyphenyl diazonium salt in 0.5 M HCl at scan rate of 50 mVs^{-1} on CNF-modified electrodes (A), Raman spectra of the CNF- electrode before and after diazonium reduction using 3 CV scans (B) and XPS C1s high resolution spectra of the CNF-modified electrodes before (C) and after (D) carboxyphenyl electrografting.

Fig. 3. Square wave voltammetry in ($K_3Fe(CN)_6/K_4Fe(CN)_6$) redox couple of the bare CNF electrodes (black) after electrografting using 3 CV scans (red) and after immobilization of SMN1 (green), CFTR (blue) and DMD (cyan) antibodies.

Fig. 4 Square wave voltammograms of the multiplexed immunosensor before and after binding with different concentrations of the three specific proteins. (A) is the SMN1 immunosensor, (B) is the CFTR immunosensor and (C) is the DMD immunosensor, the calibration curves for SMN1 (D), CFTR (E) and DMD (F) detection on the multiplexed immunosensor (plot of logarithm of the protein concentration versus the change in the square wave voltammetry peak current before and after binding $(i - i^o)/i^o\%$. The error bars represent the standard deviations of three measurements.

Fig. 5 The immunosensor response for 1 ng/mL of SMN1, CFTR and DMD against control electrode (A) and immunosensor responses to the simultaneous detection of SMN1, CFTR and

DMD (B); with addition of a mixture of 1 ng/ml SMN1+CFTR+DMD; SMN1+CFTR; and DMD alone). SMN protein levels in SMA patient and control blood samples (C), CFTR protein levels in CF patient and control blood samples and (D) DMD protein levels in DMD patient and control blood samples.

Scheme 1 multiplexed detection scheme: (A) Schematic diagram of the array electrodes on which the three antibodies and BSA (as control) were immobilized. (B) functionalization scheme of the CNF electrodes by diazonium salt electroreduction and immunosensor fabrication.

Fig. 1

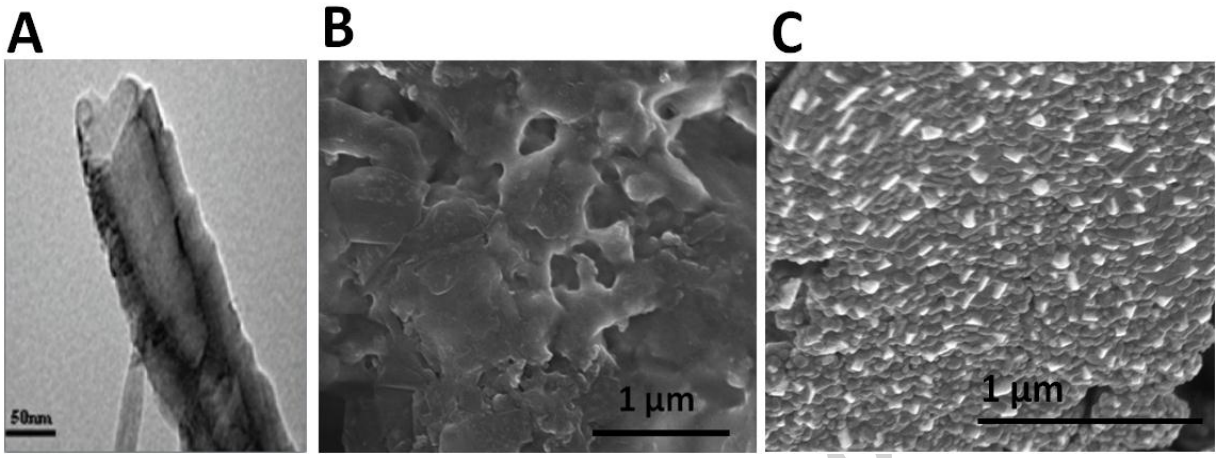


Fig. 2

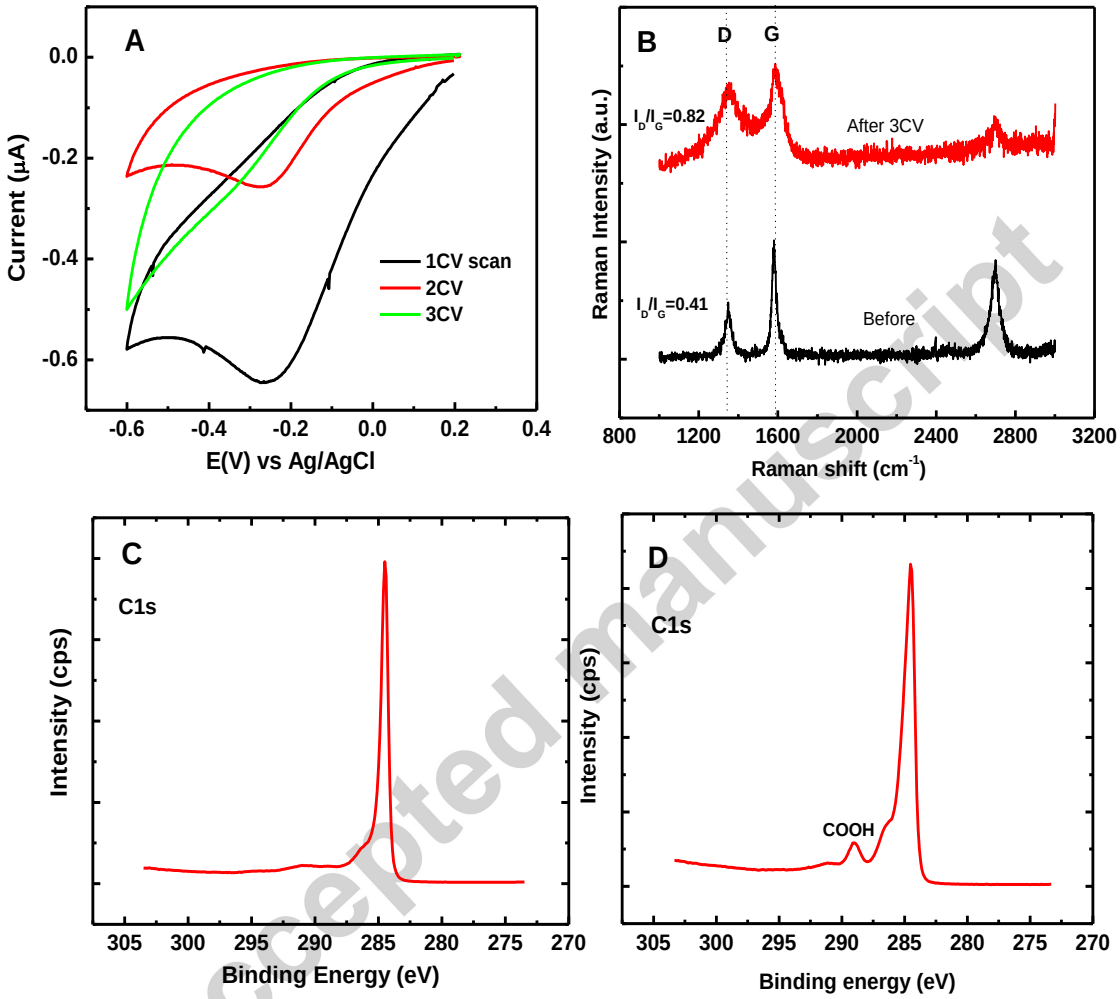


Fig. 3

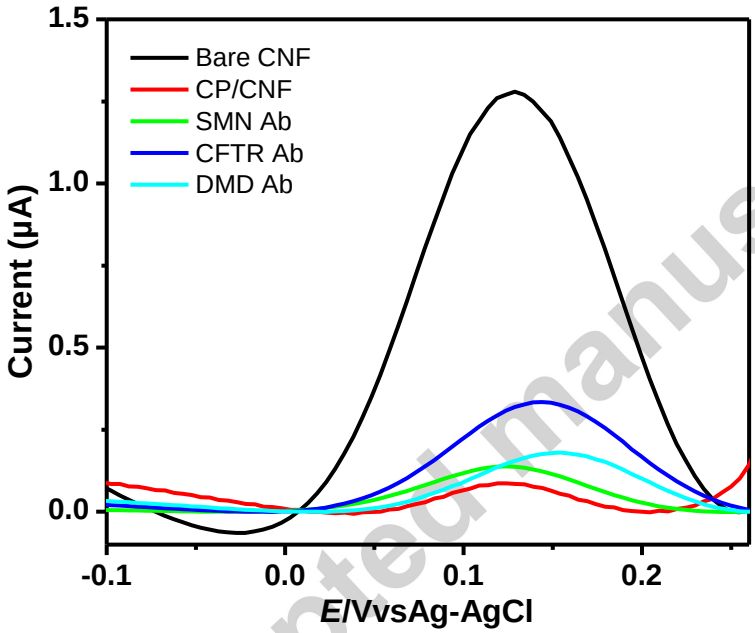


Fig. 4

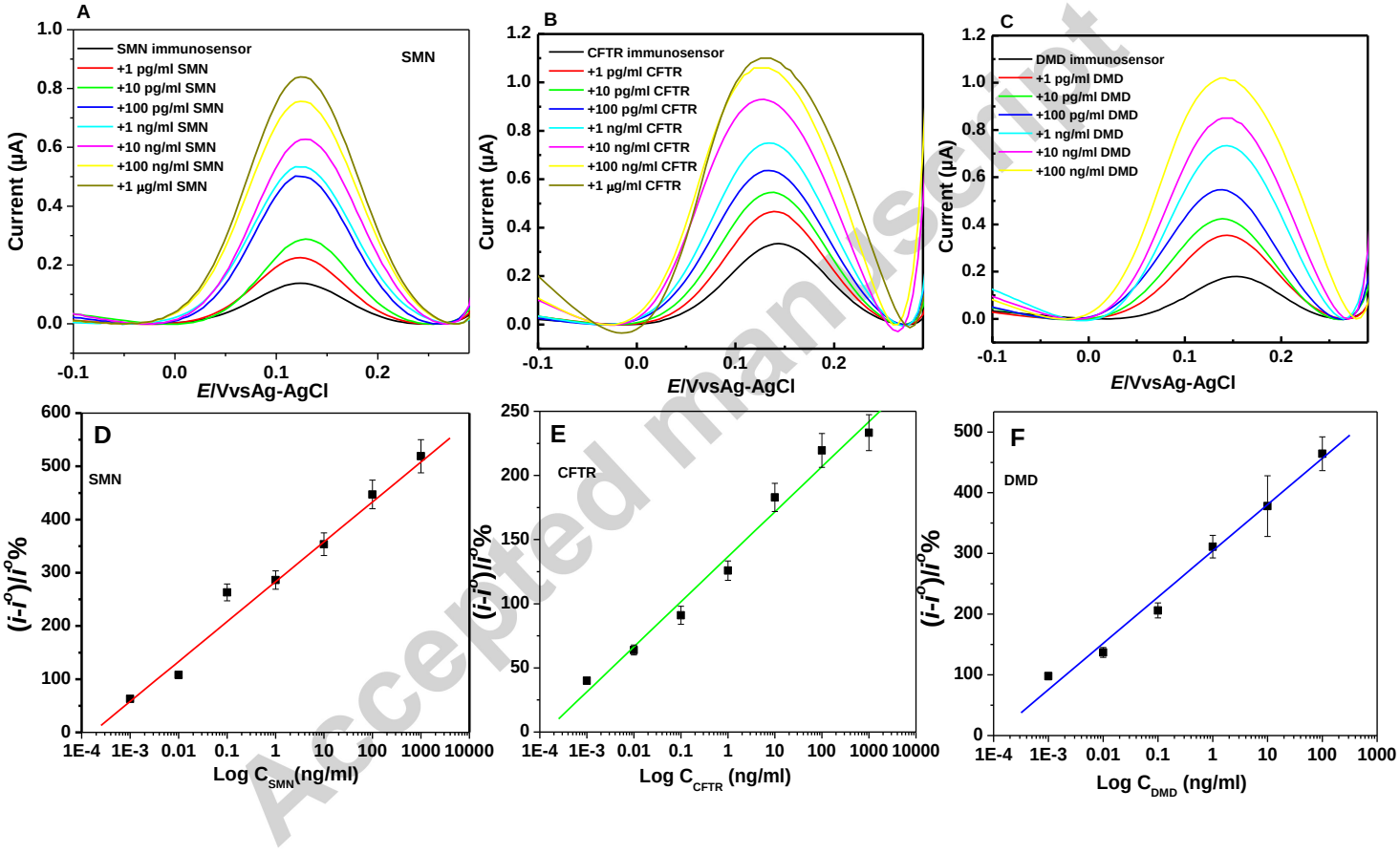
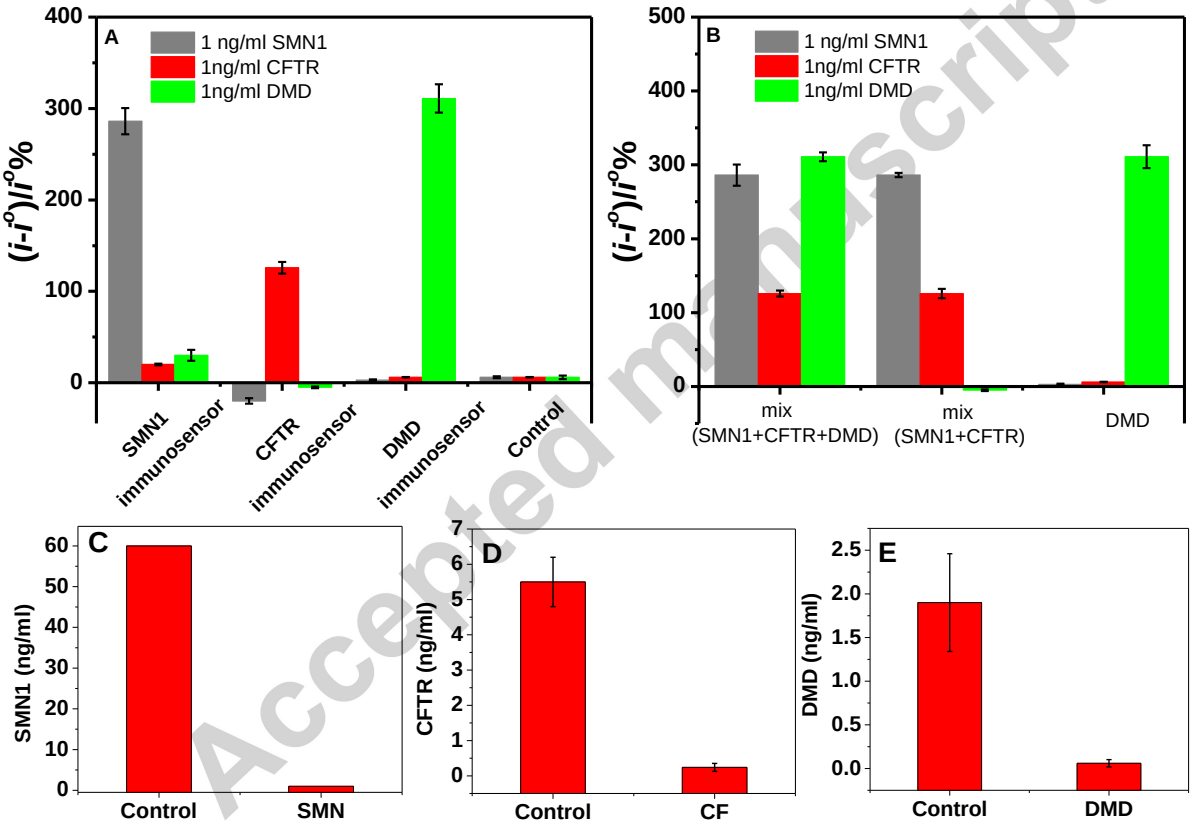
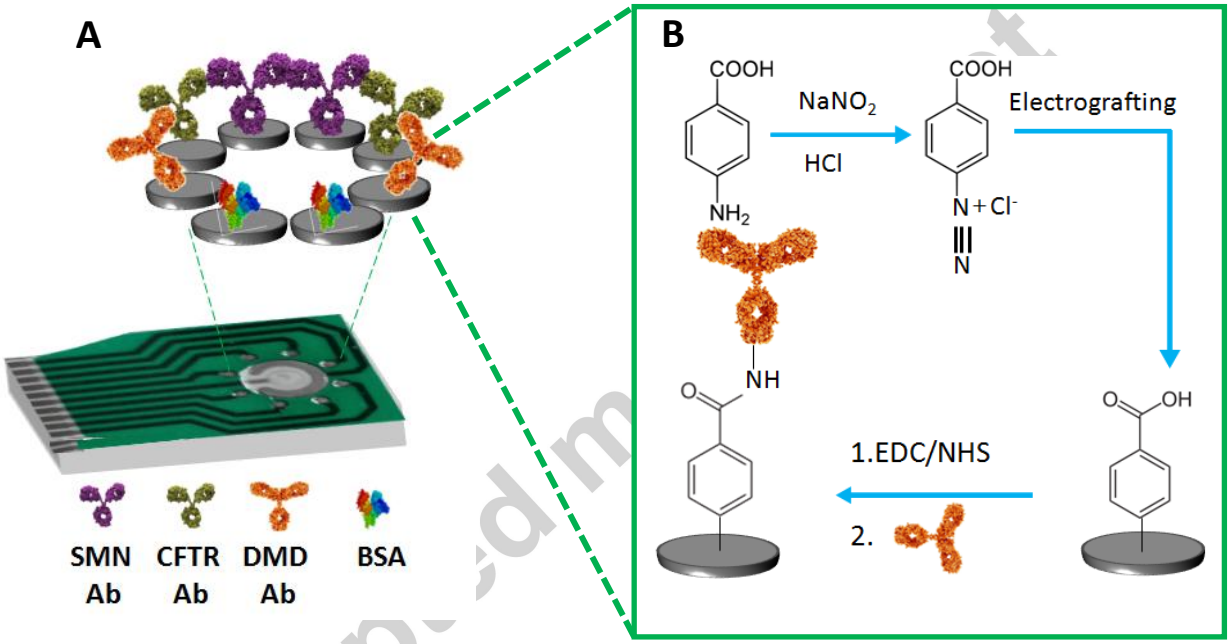


Fig. 5



Scheme1



Highlights

- Simultaneous and point-of-care detection of multiple protein biomarkers has significant impact on patient care.
- Spinal Muscular Atrophy (SMA), Cystic Fibrosis (CF) and Duchenne Muscular Dystrophy (DMD) are well known progressive hereditary disorders associated with increased morbidity as well as mortality.
- Disposable carbon nanofiber (CNF)-based electrochemical immunosensor for simultaneous detection of the detection of SMN1, CFTR and DMD proteins.
- Detection limit of CFTR and DMD and SMN1 were as low as 0.9 pg/ml, 0.7 pg/ml and 0.74 pg/ml, respectively.
- The multiplexed immunosensor has also shown strong selectivity against non-specific proteins.