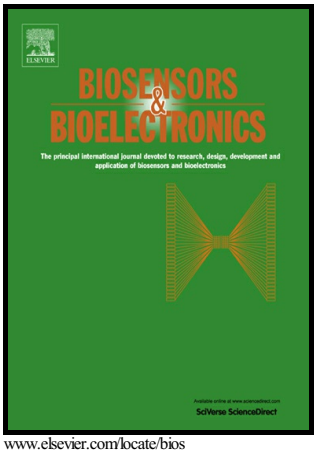


www.elsevier.com/locate/bios

Conducting electrospun fibres with polyanionic grafts as highly selective, label-free, electrochemical biosensor with a low detection limit for non-Hodgkin lymphoma gene

Thomas E. Kerr-Phillips, Nihan Aydemir, Eddie Wai Chi Chan, David Barker, Jenny Malmstrom, Cedric Plesse, Jadranka Travas Sejdic

PII: S0956-5663(17)30650-4
DOI: https://doi.org/10.1016/j.bios.2017.09.042
Reference: BIOS10016

To appear in: *Biosensors and Bioelectronic*

Received date: 19 July 2017
Revised date: 21 September 2017
Accepted date: 25 September 2017

Cite this article as: Thomas E. Kerr-Phillips, Nihan Aydemir, Eddie Wai Chi Chan, David Barker, Jenny Malmstrom, Cedric Plesse and Jadranka Travas Sejdic, Conducting electrospun fibres with polyanionic grafts as highly selective, label-free, electrochemical biosensor with a low detection limit for non-Hodgkin lymphoma gene, *Biosensors and Bioelectronic,* https://doi.org/10.1016/j.bios.2017.09.042

Conducting electrospun fibres with polyanionic grafts as highly selective, label-free, electrochemical biosensor with a low detection limit for non-Hodgkin lymphoma gene

Thomas E. Kerr-Phillips[a], Nihan Aydemir[a], Eddie Wai Chi Chan[a], David Barker[a], Jenny Malmstrom[a, b], Cedric Plesse[c], Jadranka Travas Sejdic[a, d, *]

[a]Polymer Electronics Research Centre (PERC), School of Chemical Sciences, University of Auckland, 23 Symonds Street, Auckland, New Zealand.

[b]Chemical and Materials Engineering, University of Auckland, 2-6 Park Avenue, Auckland, New Zealand

[c]LPPI-EA2528, Institut des Materiaux, 5 mail Gay Lussac, Neuville sur Oise, Cergy-Pontoise cedex 95031, France.

[d]MacDiarmid Institute for Advanced Materials and Nanotechnology, Victoria University of Wellington PO Box 600, Wellington, New Zealand.

KEYWORDS: Electrospinning, surface grafting, DNA Sensor, enhanced selectivity

ABSTRACT: A highly selective, label-free sensor for the non-Hodgkin lymphoma gene, with an aM detection limit, utilizing electrochemical impedance spectroscopy (EIS) is presented. The sensor consists of a conducting electrospun fibre mat, surface-grafted with poly(acrylic acid) (PAA) brushes and a conducting polymer sensing element with covalently attached oligonucleotide probes. The sensor was fabricated from electrospun NBR rubber, embedded with poly(3,4-ethylenedioxythiophene) (PEDOT), followed by grafting poly(acrylic acid) brushes and then electrochemically polymerizing a conducting polymer monomer with ssDNA probe sequence pre-attached. The resulting non-Hodgkin lymphoma gene sensor showed a detection limit of 1 aM (1 x 10^{-18} mol/L), more than 400 folds lower compared to a thin-film analogue. The sensor presented extraordinary selectivity, with only 1 %, 2.7 % and 4.6 % of the signal recorded for the fully non-complimentary, T to A and G to C base mismatch oligonucleotide sequences, respectively. We suggest that such greatly enhanced selectivity is due to the presence of negatively charged carboxylic acid moieties from PAA grafts that electrostatically repel the non-complementary and mismatch DNA sequences, overcoming the non-specific binding.

1 INTRODUCTION

Biosensing, and in particular DNA sensing, is a rapidly expanding research field, with a wide range of applications, including medical diagnostics(Karimi-Maleh et al. 2016; Karimi-Maleh et al. 2015), forensics(Frascione et al. 2013), biosecurity(Muhammad-Tahir and Alocilja 2003), food quality(Amine et al. 2006; Radke and Alocilja 2005) and environmental monitoring.(Amine et al. 2006) The development is driven by an ever-increasing demand for cost effective, portable and highly sensitive sensors with rapid response times.(Kannan et al. 2011; Shi et al. 2015; Ulianas et al. 2014; Zhu et al. 2015) Current DNA detection technology generally relies on the polymerase chain reaction (PCR) in conjunction with fluorescence measurements, and contains multiple steps that are time-consuming and require specialized instruments and skilled users.(Ulianas et al. 2014; Zhu et al. 2010) Thus, the development of a new generation of DNA sensing devices explore new materials, readouts and nanofabrication techniques. The new materials utilized include: carbon nanotubes(Cheng et al. 2009), quantum dots(Diltemiz et al. 2008; Medintz et al. 2003), semiconducting nanowires(Cui et al. 2001) and conducting polymers(Peng et al. 2009; Rahman et al. 2015; Zhu et al. 2006), while the new readouts include: optical(Park et al. 2011; Srinivas et al. 2012; Zhu et al. 2010), electrical(Cui et al. 2001), electrochemical(Aydemir et al. 2017; Peng et al. 2009; Rahman et al. 2015) and others.(Buenger et al. 2012; Murakami and Maeda 2005) Further developments are driven by advances in nano-fabrication techniques(Jianrong et al. 2004), including electrospinning.(Marx et al. 2011; Yang et al. 2014) Conducting polymers (CPs) are a class of polymeric materials with unique opto-electrical properties. We and others have shown that conducting polymers make excellent biosensors, utilizing both optical and electrochemical readouts for sensing DNA, proteins and small molecular targets of biological interests, such as hormones(Guler et al. 2015; Kannan et al. 2011; Park et al. 2011; Srinivas et al. 2012; Zhu et al. 2015) Electrochemical gene sensing in particular provides a direct electrical, real-time, label-free detection method(Aydemir et al. 2015; Kannan et al. 2011), in formats amenable to miniaturization and the fabrication of portable devices. Of the electrochemical techniques, electrochemical impedance spectroscopy (EIS) is often utilized in biosensing(Aydemir et al. 2015; Gu et al. 2005; Tlili et al. 2005), as even minute changes on the electrode surface, induced by biorecognition events, affects steric and charge barriers to interfacial charge transfer on the electrodes, giving rise to strong signals.(Lisdat and Schäfer 2008) We have shown that EIS can be effectively utilized in determining kinetics of the hybridization between an oligonucleotide (ON) probe bound on CP-modified sensing electrodes and its target oligonucleotide in solution.(Kannan et al. 2011)

A further advantage of conducting polymers, underutilized in biosensing, is the ease with which they can be incorporated into composite materials(Festin et al. 2013; Yuechen et al. 2014; Zhao et al. 2015) and chemically modified by common synthetic approaches(Chan et al. 2015; Malmström et al. 2013; Strover et al. 2016), including surface functionalization through polymer brushes.(Hackett et al. ; Malmström et al. 2013; Strover et al. 2016) These CP systems afford materials with a range of physical and mechanical properties; including tailored hydrophilicity,(Malmström et al. 2013) solubility(Chan et al. 2015) and flexibility.(Festin et al. 2013) In terms of composite CP materials, conducting polymer semi-Interpenetrated Polymer Network (sIPNs) with rubbers have been fabricated and shown to make superb flexible actuators.(Festin et al. 2013; Martin et al. 2012; Yin et al. 1997) Founded on the idea of CP sIPNs, we have recently fabricated electrospun nitrile butadiene rubber (NBR) embedded with poly(3,4-ethylenedioxythiophene) (NBR/PEDOT) fibre mats that demonstrated rubber-like flexibility with good electrochemical activity, conductivities in the range of 6 S/cm and reversible pore size changes upon cyclic oxidation and reduction.(Kerr-Phillips et al. 2015) Advantages of electrospinning are the simplicity of producing porous, high surface fibre mats, and the capability of large-scale production.(Ding et al. 2010; Guler et al. 2015; Yang et al. 2014) The fabrication of high surface area mats is of particular relevance to biosensors, as the surface area plays a major role in the sensor performance.(Cui et al. 2001; Kannan et al. 2012; Luo et al. 2006)

In this work, we advantageously combine specific functionalization of CPs via polymer side-chain grafting with our electrospun NBR/PEDOT s-IPNs fibre mats, to create a DNA sensor with a very low detection limit, in the aM concentration range for the target oligonucleotide, and an exceptional selectivity. Each component of the sensor contributes uniquely to that outcome. The sensing fibre mats were formed as follows. The electrospun NBR rubber fibre mats were firstly embedded with PEDOT, followed by electropolymerising a CP monomer functionalized with a polymerisation initiating site.(Malmström et al. 2013) Poly(acrylic acid) brushes were then grafted from the initiation sites and finally a single stranded oligonucleotide (ssON) probe – pre-attached to a conducting polymer monomer – was electrochemically polymerized onto the fibres. The fabricated electrochemical sensor was hydrophilic, highly porous, with a high surface area and coated with covalently bound ON probe. The probe was specific to non-Hodgkin lymphoma gene (Scheme 1). Such a sensor could be used to develop quick, accurate "liquid biopsies" that may help in early detection and avoid tissue biopsies.(Melani and Roschewski 2016). Here we demonstrate the detection limit of 1 aM, detecting ca. 600 molecules in 1 ml of buffer solution; a 400 fold improvement compared to a thin-film sensor analogue.

2 EXPERIMENTAL

2.1 Fabrication of PEDOT embedded NBR/PEGDM fibres

PEDOT embedded electrospun NBR mats were prepared as previously reported(Kerr-Phillips et al. 2015); more information can be found in SI.

2.2 Grafting of poly(acrylic acid) brushes from PBrEDOT coated fibres

Poly(acrylic acid) (PAA) brushes were produced by grafting of *tert*-butyl acrylate (tBA) by surface-initiated ARGET ATRP (activators regenerated by electron transfer atom transfer radical polymerization) (Matyjaszewski et al. 2007; Matyjaszewski and Tsarevsky 2014) and subsequently hydrolysed. For more information see SI.

2.3 Copolymerization of ThPhCONH-ON and ThPhEG

THPhCONH-ON and ThPhEG were potentiodynamically polymerized at a molar ratio 0f 1:200 onto the PAA grafted fibres; details are provided in SI.

2.4 Gene sensing measurements

For gene sensing experiments, fibre mats were cut into strips of 0.2 × 2 cm. A small piece of platinum mesh was wrapped around one end to ensure a good electrical contact and to prevent rusting of the alligator clips (**Figure S8**).

Gene sensing was performed using electrochemical impedance spectroscopy (EIS), with a Biologic SP300 potentiostat. The samples were incubated in the target oligonucleotide solutions in PBS (pH 7.6) (fully complementary, mismatched and a control solutions with no ONs) at 42 °C for one hour and the measurements were performed before and after exposing the sensing mats to solutions containing oligonucleotide targets at increasing concentrations from 1 aM to 100 pM. After incubation the samples were thoroughly washed with PBS (pH 7.6). EIS experiments were performed in PBS (pH 7.6) containing $K_3[Fe(CN)_6]/K_4(CN)_6$ redox couple (5 mM each). The impedance data were measured and collected at harmonic frequencies from 0.1 Hz to 100 kHz, with 10 mV sinusoidal excitation amplitude at an applied bias potential of 0.23 V (vs. Ag/AgCl, 3 M KCl), the EIS spectra was recorded sequentially until there was negligible change in consecutive spectra, thus allowing the fibres to equilibrate with the solution

The EIS curves were fitted with a Randles equivalent circuit consisting of a resistor (R_s) in series with a constant phase element (CPE_1) that is parallel with a resistive element, R_{ct}, which is in series with another constant phase element (CPE_2), see **Figure S9 inset**. Here R_s represents the solution resistance, R_{ct} the charge transfer resistance of the conducting polymer/solution interface, with pseudo-capacitance (CPE_2) and double layer capacitance (CPE_1).(Pei et al. 2012)

3 RESULTS AND DISCUSSION

3.1 Conducting fibre mats

The fibres were electrospun from a solution containing nitrile butadiene rubber (NBR) and poly(ethylene glycol)dimethacrylate (PEGMA), using a procedure as reported by us previously.(Kerr-Phillips et al. 2015) These materials were chosen as they afford a fibre mat that is flexible, robust, thermally stable and insoluble after crosslinking. After PEDOT incorporation the resulting NBR/PEGMA/PEDOT s-IPN were free standing, flexible, easy to handle and conducting (with a conductivity of 6 ± 3 S/cm) fibre mats (**Figure S1** and **Figure 1A**).

The surface area of the PEDOT embedded fibre mats was estimated under an assumption that the mats were comprised of tubular fibres of an average diameter of 6.26 ± 2.99 µm, as estimated from SEM, and using the estimated porosity of the mats (see SI for details). The obtained estimate for the surface area was 0.64 m^2 cm^{-3}, an approximately 60 fold increase compared to a film form, which is in good agreement with literature reports on the surface area of electrospun fibres.(Greiner and Wendorff 2007; Wang et al. 2004)

3.2 Grafting of poly(acrylic acid) brushes

To enhance the hydrophilicity of the conducting sIPN fibre mats, poly(acrylic acid) (PAA) brushes were grafted from the fibre's surface. The hydrophilicity of the mats is important as the DNA sensors operates in an aqueous environment and the degree of wetting of the substrate surface may significantly affect the sensor's performance. PAA was chosen here due to its high hydrophilicity and negative charge in relevant pH's (pH > 7).(Aulich et al. 2010) The negatively charged surface is expected to repel negatively charged oligonucleotides. The hypothesis was that, if the conditions are favourable, the Watson-Crick base pairing between the surface-attached probe and the fully complementary target sequence would overcome the electrostatic repulsions, while the base-pairing for mismatched and non-complementary sequences would not.

To enable PAA brush grafting, a functionalized EDOT with initiating sites for ATRP(Malmström et al. 2013), abbreviated as BrEDOT, was electrochemically polymerized on the fibres surface (Figure 1B). The electropolymerisation of PBrEDOT was confirmed by an increase in the oxidative currents during the potentiodynamic electropolymerisation (see SI, **Figure S2**). *t*BA was then grafted from the ATRP initiating sites on the PBrEDOT and subsequently hydrolysed to poly(acrylic acid). Further information regarding the ATRP grafting can be found in SI.

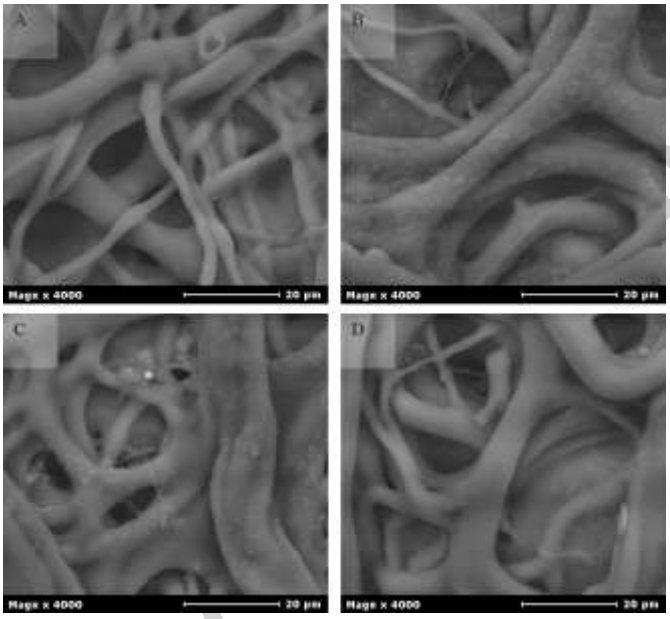


Figure 1. SEM images of: A) PEDOT embedded NBR/PEGDM electrospun fibres, B) PEDOT embedded NBR/PEGDM electrospun fibres with electrochemically deposited PBrEDOT, C) PEDOT embedded NBR/PEGDM electrospun fibres with PtBA grafts and D) PEDOT embedded NBR/PEGDM electrospun fibres with PAA grafts.

SEM images (**Figure 1**) and EDX (see SI, **Figure S10**) analysis indicates that the brushes are distributed over the entire fibre surface. Some inhomogeneity in the coverage is observed in the form of small 'clumps' on the fibre surface that originates from the commonly observed rough morphology of PEDOT (and PBrEDOT here) grown electrochemically.(Randriamahazaka et al. 1999; Tamburri et al. 2009)

3.3 Fabrication of DNA sensing mats

The steps involved in the preparation of the gene sensing fibre mats are depicted in **Scheme 1**.

The gene sensing fibre mat was realized by electrochemically copolymerizing two monomers we reported recently, **ThPhCONH-ON**(Aydemir et al. 2017) and **ThPhEG**,(Aydemir 2016; Chan et al. 2015) (**Figure 2**) onto the above described conducting fibre mats. The covalently attached oligonucleotide onto the **ThPhCOOH** monomer is a specific probe for non-Hodgkin lymphoma gene. The co-monomers were added into the polymerization solution at a feed ratio of 200:1 of ThPhEG: ThPhCONH-ON (mol/mol), respectively. The electropolymerisation CVs are presented in **Figure S7**. **ThPhEG** is used as a spacer for the **ThPhCONH-ON** in order to minimize steric hindrances to hybridization, while the ethylene glycol side chains are also expected to help

preventing eventual fouling of the sensing elements(Hackett et al. 2015; Wagner et al. 2004). Advantages of pre-attachment of the ON probe sequence onto the monomer (i.e. **ThPhCONH-ON**) were discussed elsewhere(Aydemir 2016; Chan et al. 2015) but most importantly, probe pre-attachment allows for flexibility in the choice of the oligonucleotide sequence, targeting a desired analyte. In this case, pre-attachment of ON probe onto the monomer also avoids complications that would arise from using EDC/NHS chemistry in the presence of PAA brushes that contain carboxylic acid moieties, as these would be active towards the probe ON attachment post-polymerization.

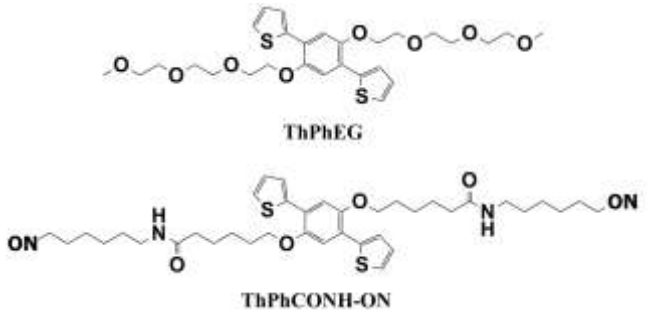
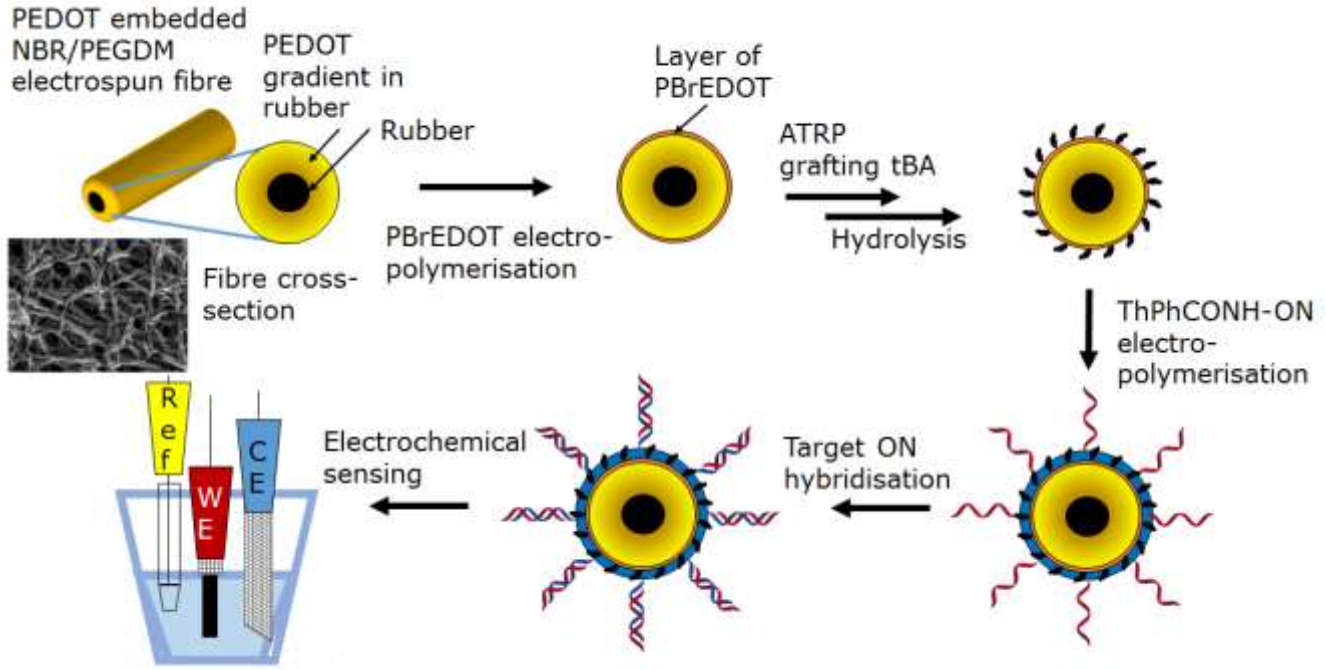


Figure 2. Monomers used in the gene sensing component of the sensor. ON = oligonucleotide.

The electrocopolymerisation of **ThPhEG** and **ThPhCONH-ON** onto the fibre mats thus produces the sensor where the target capturing and sensing element (poly(ThPhEG–co- ThPhCONH-ON) is embedded in PAA brushes and also intimately linked with the signal transducing conducting polymer layers of the fibre mats (PEDOT).

An optical image of the resulting sensor can be seen in the SI, **Figure S8**.



Scheme 1. Scheme of the fabrication of the electrospun PEDOT gene sensor.

3.4 Sensing of non-Hodgkin lymphoma gene

Non-Hodgkin lymphoma gene sensing experiments were carried out using EIS(Aydemir et al. 2017; Kannan et al. 2011) in PBS buffer containing 5 mM $[Fe(CN)_6]^{3-/4-}$ and by incrementally increasing concentrations of the target ON sequence, starting with the lowest (1 aM).(Aydemir et al. 2017; Kannan et al. 2011) The obtained EIS spectra, before and after incubation of the sensor mats in the target ON solutions, were collected and fitted with a modified Randal's equivalent circuit (**Figure S9**, inset).(Aydemir et al. 2017; Kannan et al. 2011) The Warburg impedance element in the fitting model was replaced with a constant phase element to account for a unique morphology of the substrates (i.e. multiple layers) and swelling capability of the s-IPN, complicating the diffusion. The resulting model fitted the experimental data well, as can be seen in **Figure S9**. The change in the charge transfer resistance, normalized to the charge transfer resistance of the sensor before incubation in the target solution, was taken as the signal for the hybridization.

Figure 3 presents the normalized response curve for the sensor. As can be seen, there is a negligible change in the responses when there is no target ON present in the solution (hollow points), indicating exceptional stability of the sensor over the duration of the dose-response measurements (in total ca. 10 hrs). Additionally, repeat experiments reveal the reproducibility of the sensor (n = 3) with relatively small standard deviations. Furthermore, the ThPhCONH-ON system (thin film analogue) was shown by us previously to be stable for at least two days in PBS solution at 4 °C, with negligible changes in the R_{ct}.(Aydemir et al. 2017) We attribute that stability to (i) the presence of the negatively charged grafted PAA chains (surface bound PAA has a p*K*a of ca. 4.4)(Aulich et al. 2010; Dong et al. 2009; Malmström et al. 2013) that are highly swollen in the buffer (a pH of 7.6) and prevent chemisorption of the negatively charged $[Fe(CN)_6]^{3-/4-}$ redox couple(Ding et al. 2010; Wink et al. 1997) ; and (ii) the crosslinking of the fibres make the sensor unaffected by solvents and temperature. Thus the observed stability of the fibres reinforces the choice of cross-linked NBR/PEGDM as the sensor's porous fibre mat base.

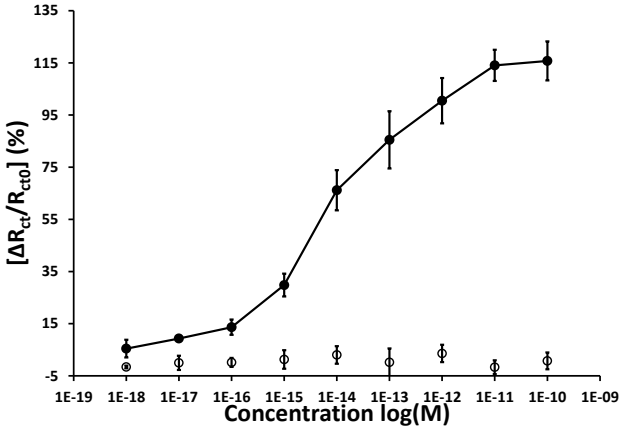


Figure 3. Sensor response (expressed as the relative change in charge transfer resistance upon hybridization) on incubation with different concentrations of the complementary ON on microfiber electrodes. Error bar taken from standard deviation, n = 3. Hollow points are negative control measured by incubation in PBS at relative intervals.

Detection limit and sensitivity of the sensor

The sensitivity of the sensor was determined to be 0.252 normalized R_{ct}/log(conc./M) (with an R^2 value of 0.9799), calculated from the slope of the linear region of the response curve, **Figure 3**. The value is comparable to that of the similar thin film electrodes (0.341 normalized R_{ct}/log(conc./M), reported by Aydemir et al. (Aydemir 2016) The detection limit, however, of the sensors is estimated to be a striking 1 aM, calculated as the concentration at which the signal to noise ratio (SNR) is greater than five(Santhosh et al. 2009), where the SNR was calculated as the change in normalised R_{CT} over the change in normalised R_{CT} for the control measurements taken without target present. The low detection limit obtained here is a ca. 400-fold improvement over the detection limit obtained with the thin film electrodes based on the same conducting copolymer(Aydemir 2016), however without the grafted PAA. We attribute such remarkable improvement to the large surface area of the sensor and the presence of charged PAA grafted side chains and the resulting heterogeneity caused by the PAA brushes. Increasing a sensor's surface area to improve its detection limit is a well-documented(Luo et al. 2006), with some recent examples from the literature that report electrospun PEDOT/PLA fibres for glucose sensing(Yang et al. 2014) and polyaniline nanowires on glassy carbon electrodes for DNA sensing(Zhu et al. 2006) or the use of nanoparticles in gas sensing.(Lü et al. 2014) A comparison of this work to other recent relevant literature reports can be found in the SI Table S3.

The sensor presents a wide dynamic range, from ca. 1 aM to 100 pM. An eight order of magnitude dynamic range in such low concentration region can be attributed to the high surface area, the PAA brushes, the surface heterogeneity and adequate spacing of sensing moieties that facilitate hybridization efficiency.(Liu and Dutton 2009; Zhu et al. 2015) We hypothesize that the excellent sensitivity for such a large mat is attributed to heterogeneity of the PAA brushes, which grow in clumps induced by the rough PBrEDOT morphology (Figure 1B), although the EDX analysis (Figure S10) suggests that the PAA brushes cover the entire fibre surface. Thus the PAA brush layer is of non-homogeneous thickness. Based on these observations, we propose that at the thinnest points the PAA layers are thin enough to effectively act as 'pinholes', to allow a limited interaction of negatively charged $[Fe(CN)_6]^{3-/4-}$ redox couple with the underlying PEDOT. Within such 'pinholes' the PAA brush layer would be less than the Debye length thick (0.76 nm in PBS(Noémie et al. 2013), approximately 3 to 5 PAA units(Jang et al. 2007)) and at least greater than twice the Debye length wide, pinholes of smaller dimensions should be effectively screened by the Debye double layer. These nanoscale pinholes would be the places where the poly(ThPhCONH-ON-co-ThPhEG) preferentially electrochemically polymerize; thus the sensor is comprised of microfibers with nano-scaled sensing regions. This would constrain the sensing elements to the small areas of the fibres, enhancing the Donnan exclusion of the redox couple(Kannan et al. 2011) and creating nano-scaled convex holes with optimised geometry for sensing,(Shoorideh and Chui 2014) therefore facilitating the low detection limit and high sensitivity. However, the reasons for such an excellent sensing response would need further investigation.

3.5 Selectivity of the sensor

To investigate the selectivity of the sensor, the sensor was incubated with a solution of fully non-complementary, T to A and G to C base mismatch DNA sequences, at a concentration of 1 pM. The normalized R_{ct} values were compared to the signal from the so-

lution of a fully complementary sequence of the same concentration (taken as 100%) (**Figure 4**). The Figure 4 shows exceptionally good selectivity of the sensor towards mismatched sequences, with 1.1 %, 2.7 % and 4.6 % of the signal recorded for the fully non-complimentary, G to C mismatched and T to A mismatch oligonucleotide sequence, respectively. The slightly higher response from the T to A mismatch sequence is excepted, based on the strength of hydrogen bonding between the bases(Szatyłowicz and Sadlej-Sosnowska 2010). We suggest that this impressive selectivity is due to the negatively charged surface of the fibres, caused by the PAA brushes that electrostatically repel DNA.(Booth et al. 2011; Peng et al. 2006; Peng et al. 2009) The non-Hodgkin lymphoma gene sequence, that is complementary to the fibre-bound probe sequence, overcome the electrostatic repulsive force though favourable intermolecular interactions with the probe ON sequence, giving rise to the signal. The use of Equation 2 may aid in understanding this finding:(Kannan et al. 2011)

$$\theta = \frac{k_{on}c}{k_{on}c + k_{off}}(1 - e^{-\frac{t}{\tau}}) \qquad (2)$$

where θ denotes the fraction of the conducting polymer sensing film pinholes on the fibres that have double stranded oligonucleotides (dsON) bound, k_{on} and k_{off} represent the rate constants for reaction of the target ON, with solution concentration c, with the surface-bound probe ssON to form surface-bound dsON and for dissociation of the surface-bound dsON, respectively. The selectivity enhancement by the PAA brushes can be explained as the presence of the carboxylic acid groups reduce k_{on} and increase k_{off}. This will cause the equilibrium of the reaction responsible for the hybridization to favour the ssON as opposed to the dsON. In the case of mismatch and non-complementary ONs, where k_{on} is smaller and k_{off} is bigger already, the presence of the carboxylic acid groups makes the reaction to strongly favour ssON to the point where θ is close to zero. Thus, the overall effect is that the sensor's selectivity is enhanced.

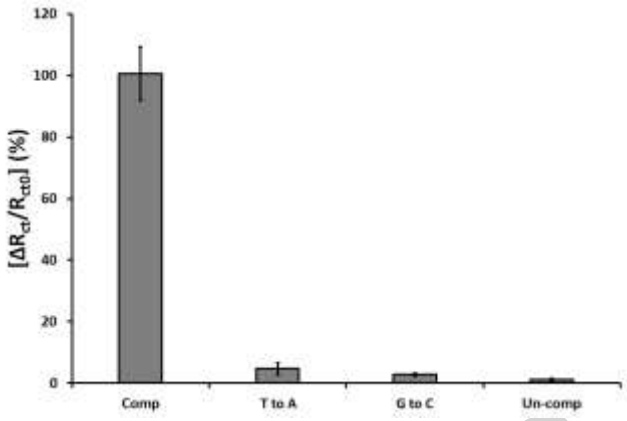


Figure 4. Normalized sensor response of the microfiber sensing electrodes after incubation in corresponding ON solutions (complementary T to A and G to C base mismatch and non-complementary sequences) at concentration of 1 pM.

4 CONCLUSION

In this study we demonstrated fabrication of a novel DNA sensor based on conductive microfibers comprised of a rubber-based semi interpenetrated network of NBR/PEGDM/PEDOT. The sensing substrate is flexible, robust and easy to handle. The sensor demonstrated an ultra-low detection limit of 1 aM for non-Hodgkin lymphoma gene, superb selectivity, even to the one-base T to A mismatched target (4.6 % of the signal for the fully non-complimentary target), and with eight orders of magnitude dynamic sensing range. This excellent gene sensing performance is attributed to a combination of the unique materials' properties employed in constructing the sensor, i.e. the conducting polymer monomers with pre-attached ON probes, the conducting electrospun support and the PAA brushes grafted from the fibres surface. The electrospun fibres provide high surface area to volume ratio enhancing the detection limit. The PAA brushes repel mismatch ON sequences and also prevent physiosorption of the $[Fe(CN)_6]^{3-/4-}$ redox probe, both enhancing selectivity and sensitivity of the sensor.

In more general terms, we demonstrated advantageous use of the molecular design of materials in highly sensitive and selective, label-free, electrochemical biosensing.

ASSOCIATED CONTENT

Experimental details and further supporting information can be found in Supporting Information.

AUTHOR INFORMATION

Corresponding Author

* Prof. Jadranka Travas-Sejdic: j.travas-sejdic@auckland.ac.nz

Funding Sources

Thomas Kerr-Phillips would like to acknowledge the MacDiarmid Institute for Advanced Materials and Nanotechnology or his PhD scholarship.

ACKNOWLEDGMENT

The authors would like to acknowledge Prof. David Williams for his insightful contributions to the interpretation of the impedance results.

REFERENCES

Amine, A., Mohammadi, H., Bourais, I., Palleschi, G., 2006. Enzyme inhibition-based biosensors for food safety and environmental monitoring. Biosensors and Bioelectronics 21(8), 1405-1423.

Aulich, D., Hoy, O., Luzinov, I., Brücher, M., Hergenröder, R., Bittrich, E., Eichhorn, K.-J., Uhlmann, P., Stamm, M., Esser, N., Hinrichs, K., 2010. In Situ Studies on the Switching Behavior of Ultrathin Poly(acrylic acid) Polyelectrolyte Brushes in Different Aqueous Environments. Langmuir 26(15), 12926-12932.

Aydemir, N., 2016. Development of highly sensitive and selective DNA sensors based on conducting polymers. School of Chemical Sicences, p. 246. the University of Auckland.

Aydemir, N., Chan, E., Baek, P., Barker, D., Williams, D.E., Travas-Sejdic, J., 2017. New immobilisation method for oligonucleotides on electrodes enables highly-sensitive, electrochemical label-free gene sensing. Biosensors and Bioelectronics 97, 128-135.

Aydemir, N., McArdle, H., Patel, S., Whitford, W., Evans, C.W., Travas-Sejdic, J., Williams, D.E., 2015. A Label-Free, Sensitive, Real-Time, Semiquantitative Electrochemical Measurement Method for DNA Polymerase Amplification (ePCR). Analytical Chemistry 87(10), 5189-5197.

Booth, M.A., Harbison, S., Travas-Sejdic, J., 2011. Development of an electrochemical polypyrrole-based DNA sensor and subsequent studies on the effects of probe and target length on performance. Biosensors and Bioelectronics 28(1), 362-367.

Buenger, D., Topuz, F., Groll, J., 2012. Hydrogels in sensing applications. Progress in Polymer Science 37(12), 1678-1719.

Chan, E.W.C., Baek, P., Barker, D., Travas-Sejdic, J., 2015. Highly functionalisable polythiophene phenylenes. Polymer Chemistry 6(43), 7618-7629.

Cheng, Y., Liu, Y., Huang, J., Li, K., Xian, Y., Zhang, W., Jin, L., 2009. Amperometric tyrosinase biosensor based on Fe3O4 nanoparticles-coated carbon nanotubes nanocomposite for rapid detection of coliforms. Electrochimica Acta 54(9), 2588-2594.

Cui, Y., Wei, Q., Park, H., Lieber, C.M., 2001. Nanowire Nanosensors for Highly Sensitive and Selective Detection of Biological and Chemical Species. Science 293(5533), 1289-1292.

Diltemiz, S.E., Say, R., Büyüktiryaki, S., Hür, D., Denizli, A., Ersöz, A., 2008. Quantum dot nanocrystals having guanosine imprinted nanoshell for DNA recognition. Talanta 75(4), 890-896.

Ding, Y., Wang, Y., Li, B., Lei, Y., 2010. Electrospun hemoglobin microbelts based biosensor for sensitive detection of hydrogen peroxide and nitrite. Biosensors and Bioelectronics 25(9), 2009-2015.

Dong, R., Lindau, M., Ober, C.K., 2009. Dissociation Behavior of Weak Polyelectrolyte Brushes on a Planar Surface. Langmuir 25(8), 4774-4779.

Festin, N., Maziz, A., Plesse, C., Teyssié, D., Chevrot, C., Vidal, F., 2013. Robust solid polymer electrolyte for conducting IPN actuators. Smart Materials and Structures 22(10), 104005.

Frascione, N., Gooch, J., Daniel, B., 2013. Enabling fluorescent biosensors for the forensic identification of body fluids. Analyst 138(24), 7279-7288.

Greiner, A., Wendorff, J.H., 2007. Electrospinning: A Fascinating Method for the Preparation of Ultrathin Fibers. Angewandte Chemie International Edition 46(30), 5670-5703.

Gu, H., Su, X.d., Loh, K.P., 2005. Electrochemical Impedance Sensing of DNA Hybridization on Conducting Polymer Film-Modified Diamond. The Journal of Physical Chemistry B 109(28), 13611-13618.

Guler, Z., Erkoc, P., Sarac, A.S., 2015. Electrochemical impedance spectroscopic study of single-stranded DNA-immobilized electroactive polypyrrole-coated electrospun poly(ε-caprolactone) nanofibers. Materials Express 5(4), 269-279.

Hackett, A.J., Malmstrom, J., Molino, P.J., Gautrot, J.E., Zhang, H., Higgins, M.J., Wallace, G.G., Williams, D.E., Travas-Sejdic, J., 2015. Conductive surfaces with dynamic switching in response to temperature and salt. Journal of Materials Chemistry B 3(48), 9285-9294.

Hackett, A.J., Malmström, J., Travas-Sejdic, J., Functionalization of conducting polymers for biointerface applications. Progress in Polymer Science.

Jang, S.S., Goddard, W.A., Kalani, M.Y.S., 2007. Mechanical and Transport Properties of the Poly(ethylene oxide)−Poly(acrylic acid) Double Network Hydrogel from Molecular Dynamic Simulations. The Journal of Physical Chemistry B 111(7), 1729-1737.

Jianrong, C., Yuqing, M., Nongyue, H., Xiaohua, W., Sijiao, L., 2004. Nanotechnology and biosensors. Biotechnology Advances 22(7), 505-518.

Kannan, B., Williams, D.E., Booth, M.A., Travas-Sejdic, J., 2011. High-Sensitivity, Label-Free DNA Sensors Using Electrochemically Active Conducting Polymers. Analytical Chemistry 83(9), 3415-3421.

Kannan, B., Williams, D.E., Laslau, C., Travas-Sejdic, J., 2012. A highly sensitive, label-free gene sensor based on a single conducting polymer nanowire. Biosensors and Bioelectronics 35(1), 258-264.

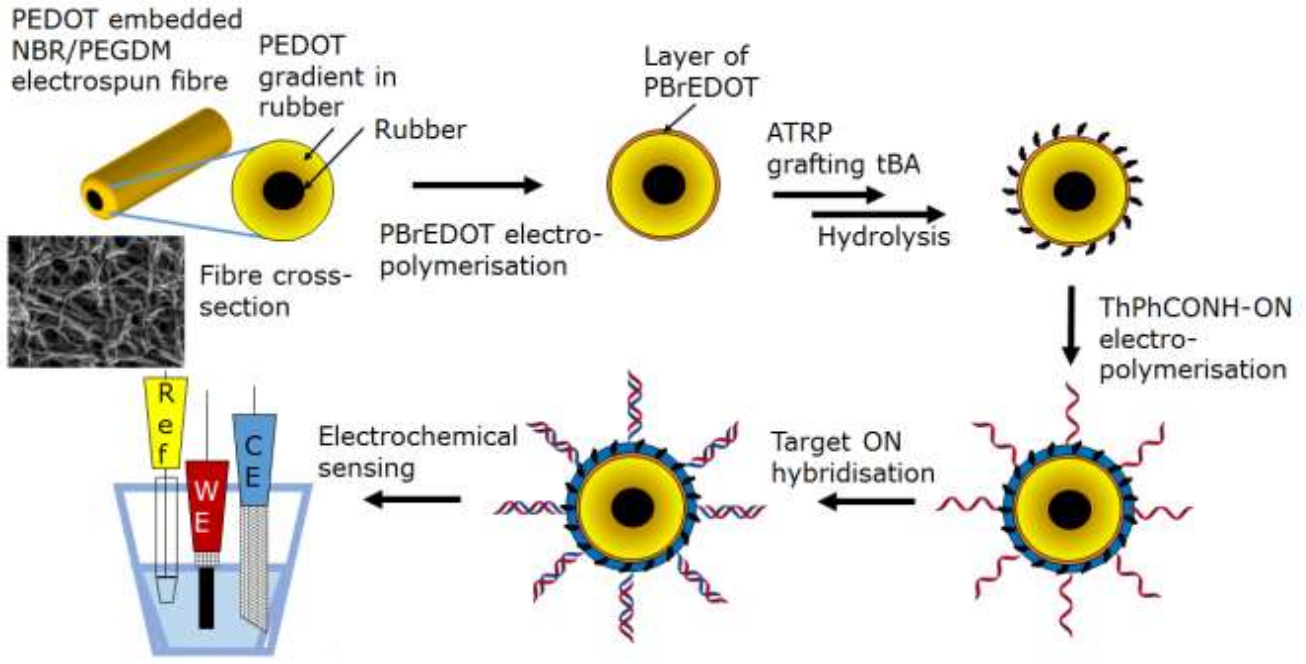
Karimi-Maleh, H., Shojaei, A.F., Tabatabaeian, K., Karimi, F., Shakeri, S., Moradi, R., 2016. Simultaneous determination of 6-mercaptopruine, 6-thioguanine and dasatinib as three important anticancer drugs using nanostructure voltammetric sensor employing Pt/MWCNTs and 1-butyl-3-methylimidazolium hexafluoro phosphate. Biosensors and Bioelectronics 86, 879-884.

Karimi-Maleh, H., Tahernejad-Javazmi, F., Atar, N., Yola, M.L., Gupta, V.K., Ensafi, A.A., 2015. A Novel DNA Biosensor Based on a Pencil Graphite Electrode Modified with Polypyrrole/Functionalized Multiwalled Carbon Nanotubes for Determination of 6-Mercaptopurine Anticancer Drug. Industrial & Engineering Chemistry Research 54(14), 3634-3639.

Kerr-Phillips, T.E., Woehling, V., Agniel, R., Nguyen, G.T., Vidal, F., Kilmartin, P., Plesse, C., Travas-Sejdic, J., 2015. Electrospun rubber fibre mats with electrochemically controllable pore sizes. Journal of Materials Chemistry B 3(20), 4249-4258.

Lisdat, F., Schäfer, D., 2008. The use of electrochemical impedance spectroscopy for biosensing. Analytical and Bioanalytical Chemistry 391(5), 1555.
Liu, Y., Dutton, R.W., 2009. Effects of charge screening and surface properties on signal transduction in field effect nanowire biosensors. Journal of Applied Physics 106(1), 014701.
Lü, Y., Zhan, W., He, Y., Wang, Y., Kong, X., Kuang, Q., Xie, Z., Zheng, L., 2014. MOF-Templated Synthesis of Porous Co3O4 Concave Nanocubes with High Specific Surface Area and Their Gas Sensing Properties. ACS Applied Materials & Interfaces 6(6), 4186-4195.
Luo, X., Morrin, A., Killard, A.J., Smyth, M.R., 2006. Application of Nanoparticles in Electrochemical Sensors and Biosensors. Electroanalysis 18(4), 319-326.
Malmström, J., Nieuwoudt, M.K., Strover, L.T., Hackett, A., Laita, O., Brimble, M.A., Williams, D.E., Travas-Sejdic, J., 2013. Grafting from Poly(3,4-ethylenedioxythiophene): A Simple Route to Versatile Electrically Addressable Surfaces. Macromolecules 46(12), 4955-4965.
Malmström, J., Nieuwoudt, M.K., Strover, L.T., Hackett, A., Laita, O., Brimble, M.A., Williams, D.E., Travas-Sejdic, J., 2013. Grafting from poly (3, 4-ethylenedioxythiophene): a simple route to versatile electrically addressable surfaces. Macromolecules 46(12), 4955-4965.
Martin, B.D., Justin, G.A., Moore, M.H., Naciri, J., Mazure, T., Melde, B.J., Stroud, R.M., Ratna, B., 2012. An Elastomeric Poly(Thiophene-EDOT) Composite with a Dynamically Variable Permeability Towards Organic and Water Vapors. Advanced Functional Materials 22(15), 3116-3127.
Marx, S., Jose, M.V., Andersen, J.D., Russell, A.J., 2011. Electrospun gold nanofiber electrodes for biosensors. Biosensors and Bioelectronics 26(6), 2981-2986.
Matyjaszewski, K., Dong, H., Jakubowski, W., Pietrasik, J., Kusumo, A., 2007. Grafting from Surfaces for "Everyone": ARGET ATRP in the Presence of Air. Langmuir 23(8), 4528-4531.
Matyjaszewski, K., Tsarevsky, N.V., 2014. Macromolecular Engineering by Atom Transfer Radical Polymerization. Journal of the American Chemical Society 136(18), 6513-6533.
Medintz, I.L., Clapp, A.R., Mattoussi, H., Goldman, E.R., Fisher, B., Mauro, J.M., 2003. Self-assembled nanoscale biosensors based on quantum dot FRET donors. Nat Mater 2(9), 630-638.
Melani, C., Roschewski, M., 2016. Molecular monitoring of cell-free circulating tumor DNA in non-Hodgkin lymphoma. Oncology 30(8), 731-731.
Muhammad-Tahir, Z., Alocilja, E.C., 2003. A conductometric biosensor for biosecurity. Biosensors and Bioelectronics 18(5), 813-819.
Murakami, Y., Maeda, M., 2005. DNA-Responsive Hydrogels That Can Shrink or Swell. Biomacromolecules 6(6), 2927-2929.
Noémie, L., Rune, S.F., Thor, C.M., Nathalie, I.R., Shivendra, U., Luca De, V., Jan, H.J., Jesper, N., Karen, L.M., 2013. Effects of buffer composition and dilution on nanowire field-effect biosensors. Nanotechnology 24(3), 035501.
Park, D.H., Kim, N., Cui, C., Hong, Y.K., Kim, M.S., Yang, D.-H., Kim, D.-C., Lee, H., Kim, J., Ahn, D.J., Joo, J., 2011. DNA detection using a light-emitting polymer single nanowire. Chemical Communications 47(28), 7944-7946.
Pei, Y., Travas-Sejdic, J., Williams, D.E., 2012. Reversible Electrochemical Switching of Polymer Brushes Grafted onto Conducting Polymer Films. Langmuir 28(21), 8072-8083.
Peng, H., Soeller, C., Cannell, M.B., Bowmaker, G.A., Cooney, R.P., Travas-Sejdic, J., 2006. Electrochemical detection of DNA hybridization amplified by nanoparticles. Biosensors and Bioelectronics 21(9), 1727-1736.
Peng, H., Zhang, L., Soeller, C., Travas-Sejdic, J., 2009. Conducting polymers for electrochemical DNA sensing. Biomaterials 30(11), 2132-2148.
Radke, S.M., Alocilja, E.C., 2005. A high density microelectrode array biosensor for detection of E. coli O157:H7. Biosensors and Bioelectronics 20(8), 1662-1667.
Rahman, M., Li, X.-B., Lopa, N., Ahn, S., Lee, J.-J., 2015. Electrochemical DNA Hybridization Sensors Based on Conducting Polymers. Sensors 15(2), 3801.
Randriamahazaka, H., Noël, V., Chevrot, C., 1999. Nucleation and growth of poly(3,4-ethylenedioxythiophene) in acetonitrile on platinum under potentiostatic conditions. Journal of Electroanalytical Chemistry 472(2), 103-111.
Santhosh, P., Manesh, K.M., Uthayakumar, S., Komathi, S., Gopalan, A.I., Lee, K.P., 2009. Fabrication of enzymatic glucose biosensor based on palladium nanoparticles dispersed onto poly(3,4-ethylenedioxythiophene) nanofibers. Bioelectrochemistry 75(1), 61-66.
Shi, P., Geng, S., Li, T.-t., Li, Y.-s., Feng, T., Wu, H.-n., 2015. Methods to detect avian influenza virus for food safety surveillance. Journal of Integrative Agriculture 14(11), 2296-2308.
Shoorideh, K., Chui, C.O., 2014. On the origin of enhanced sensitivity in nanoscale FET-based biosensors. Proceedings of the National Academy of Sciences 111(14), 5111-5116.
Srinivas, A.R.G., Peng, H., Barker, D., Travas-Sejdic, J., 2012. Switch on or switch off: An optical DNA sensor based on poly(p-phenylenevinylene) grafted magnetic beads. Biosensors and Bioelectronics 35(1), 498-502.
Strover, L.T., Malmström, J., Travas-Sejdic, J., 2016. Graft Copolymers with Conducting Polymer Backbones: A Versatile Route to Functional Materials. The Chemical Record 16(1), 393-418.
Szatyłowicz, H., Sadlej-Sosnowska, N., 2010. Characterizing the Strength of Individual Hydrogen Bonds in DNA Base Pairs. Journal of Chemical Information and Modeling 50(12), 2151-2161.
Tamburri, E., Orlanducci, S., Toschi, F., Terranova, M.L., Passeri, D., 2009. Growth mechanisms, morphology, and electroactivity of PEDOT layers produced by electrochemical routes in aqueous medium. Synthetic Metals 159(5–6), 406-414.
Tlili, C., Korri-Youssoufi, H., Ponsonnet, L., Martelet, C., Jaffrezic-Renault, N.J., 2005. Electrochemical impedance probing of DNA hybridisation on oligonucleotide-functionalised polypyrrole. Talanta 68(1), 131-137.
Ulianas, A., Heng, L.Y., Ahmad, M., Lau, H.-Y., Ishak, Z., Ling, T.L., 2014. A regenerable screen-printed DNA biosensor based on acrylic microsphere–gold nanoparticle composite for genetically modified soybean determination. Sensors and Actuators B: Chemical 190, 694-701.
Wagner, V.E., Koberstein, J.T., Bryers, J.D., 2004. Protein and bacterial fouling characteristics of peptide and antibody decorated surfaces of PEG-poly(acrylic acid) co-polymers. Biomaterials 25(12), 2247-2263.

Wang, X., Kim, Y.-G., Drew, C., Ku, B.-C., Kumar, J., Samuelson, L.A., 2004. Electrostatic Assembly of Conjugated Polymer Thin Layers on Electrospun Nanofibrous Membranes for Biosensors. Nano Letters 4(2), 331-334.
Wink, T., J. van Zuilen, S., Bult, A., P. van Bennekom, W., 1997. Self-assembled Monolayers for Biosensors. Analyst 122(4), 43R-50R.
Yang, G., Kampstra, K.L., Abidian, M.R., 2014. High Performance Conducting Polymer Nanofiber Biosensors for Detection of Biomolecules. Advanced Materials 26(29), 4954-4960.
Yin, W., Li, J., Li, Y., Wu, Y., Gu, T., Liu, C., 1997. Conducting IPN Based on Polyaniline and Crosslinked Cellulose. Polymer International 42(3), 276-280.
Yuechen, L., Ryo, T., Hidenori, O., 2014. Soft and flexible PEDOT/PSS films for applications to soft actuators. Smart Materials and Structures 23(7), 074010.
Zhao, G., Sun, Z., Wang, J., Xu, Y., Muhammad, F., 2015. Development of biocompatible polymer actuator consisting of biopolymer chitosan, carbon nanotubes, and an ionic liquid. Polymer Composites, n/a-n/a.
Zhu, B., Alsager, O.A., Kumar, S., Hodgkiss, J.M., Travas-Sejdic, J., 2015. Label-free electrochemical aptasensor for femtomolar detection of 17β-estradiol. Biosensors and Bioelectronics 70, 398-403.
Zhu, D., Liu, J., Tang, Y., Xing, D., 2010. A reusable DNA biosensor for the detection of genetically modified organism using magnetic bead-based electrochemiluminescence. Sensors and Actuators B: Chemical 149(1), 221-225.
Zhu, N., Chang, Z., He, P., Fang, Y., 2006. Electrochemically fabricated polyaniline nanowire-modified electrode for voltammetric detection of DNA hybridization. Electrochimica Acta 51(18), 3758-3762.



Graphical Abstract

Highlights

- A sensitive, low detection limit, DNA sensor is applied to electrospun conducting polymer fibres.
- The selectivity is greatly enhanced by poly(acrylic acid) brushes grafted from the fibres surface before DNA sensing element is introduced.
- Sensitivity and detection limit is enhanced by the high surface area and we also propose due to the unique morphology induced by the non-homogeneous poly(acrylic acid) graft layer.