Journal of Materials Chemistry B

Intrinsically Conducting Polymer Nanowires for Biosensing

Journal:	*Journal of Materials Chemistry B*
Manuscript ID:	TB-FEA-04-2014-000598.R1
Article Type:	Feature Article
Date Submitted by the Author:	23-May-2014
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ARTICLE

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_Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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Nanomaterials are commonly exploited to increase the sensitivity of sensors. Conductive polymers are emerging as promising sensing materials as they are easy to functionalize with the appropriate sensing probes, and also act as signal transducers. By constraining the material into one dimensional nanowires, extraordinary sensitivity is achieved. This review deals with the fabrication of these electrically conductive polymer nanowire (ECPNW) sensors and their use for detecting nucleic acid sequences, proteins and pathogens.

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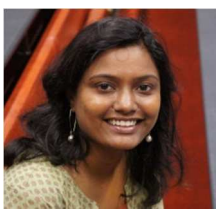

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1. Introduction

Detection of biomolecules has been of ever-increasing importance in healthcare, environmental monitoring, agricultural and aquaculture fields, food quality and biosecurity. Biosensors enable detection of genetic abnormalities, pathogens, viruses, toxins and biological markers of diseases.[1] There has been a tremendous amount of research into the development of sensitive, selective, robust, portable and cost effective biosensors, based on advanced electrical,[1-6] optical,[7-11] piezoelectric[12, 13] and magnetic[14-16] readouts. In general terms, biosensing relies on a highly specific recognition event between a probe and its target analyte. A biosensor should facilitate the formation of a highly specific probe-target complex in a way that the complex formation triggers a usable readout signal.[17] Recent advances in nano-scale materials and nanotechnology have encouraged significant amount of research into utilisation of nano-materials in sensing devices, including biosensors,[18] and of how to integrate them into microelectronic[19] and microfluidic[20] devices that would provide reliable, in-field, detection. Nano-scale materials offer the unique advantage of possessing intrinsically high surface area - a prerequisite for high sensitivity. However, in spite of significant advances achieved in recent years,[21] challenges such as achieving high specificity and ultra-high sensitivity important for early detection of diseases without false positives due to interference substances, reproducibility and production scalability, have been restraining the transitions from proof of principle to actual devices.[22] Various nano-scale materials, such as silicon nanowires (SNWs),[23, 24] carbon nanotubes (CNTs),[25, 26] inorganic semiconductor nanoparticles (Quantum Dots),[27] metallic and magnetic nanoparticles[28, 29] and electrically conducting polymer nanowires (ECPNWs)[30, 31] have been developed in order to address the above-mentioned demands.

Electrically conductive polymers (ECPs), with their unique electronic structure[32, 33] that is highly sensitive to changes in the polymeric environment and other perturbations in the chain conformation have been widely investigated in chemical sensing,[34] gas sensing[35] and biosensing.[36] The rich synthetic chemistry of ECPs can be employed to design ECPs with the appropriate chemical structure for a particular sensing application[1, 32] and various fabrication methods can be applied to obtain the desired material morphology[32, 37]. Also, there have been a number of recent studies demonstrating the biocompatibility[38, 39] and environmental stability[40, 41] of ECPs. In sensing applications, ECPs are conveniently utilised as both sensing elements and transducers.[30] Similar to other nano-scale materials, nano-sized ECPs in sensing and biosensing offer high sensitivity and the possibility of fabricating array sensing devices.[30, 42-45] In addition to relatively simple fabrication techniques accessible (as outlined below) that overcome problems found in other types of nano-materials (such as Si NW and CNT) – for example, positioning of sensing elements between microelectrodes in a device – simple synthesis methodologies, high porosity of ECPs, electrical conductivity, number and ease of bioprobe immobilisation strategies, and un-hostile organic environment for biomolecules, make these materials particularly attractive for developing novel biosensing technologies. Nanowires of polypyrrole (PPy), polyaniline (PANI), poly(ethylene dioxythiophene) (PEDOT) and their functionalized derivatives have been exploited in biosensor applications.[30, 31, 41] Building a biosensor based on ECPNWs involves three main steps: (i) fabrication of ECPNWs via either electrochemical and/or chemical polymerisation techniques; (ii) immobilisation of an appropriate bioreceptor (recognition biomolecule probe such as DNA, antibody, aptamer, virus or enzyme) via physical entrapment, covalent attachment or an affinity interaction; and (iii) utilisation of an appropriate readout methodology.

In this review we focus on recent developments in synthesis and use of ECP nanowires in *label-free* detection of biomolecules; including nucleic acids, proteins and pathogens (viruses and bacteria). The article starts with an introduction to ECP structure and properties, followed by an overview of to date developed methods for fabrication of ECP nanowires, and then focusses on reviewing the advances in their use in biosensing of nucleic acids, proteins and pathogens (see figure 1 for a schematic illustration of applications reviewed). We conclude with a summary and outlook of future possibilities in this rapidly developing field of ECP applications.

2. ECP Structure and Properties Utilised for Sensing and Biosensing

Electrically conducting polymers are conjugated polymers with unique electrical and optical properties.[46] Since their discovery[47] there has been an enormous amount of research into both their fundamental properties and technological applications. The prospect of combining high electrical conductivity with the polymer processing properties of conventional polymers has led to development of a whole new field of polymer applications and devices, including light emitting diodes (LED),[48] photovoltaics,[32, 49] electrochromic materials,[50] anti-static coatings,[51] sensors and biosensors,[52] actuators,[53-55] drug release agents,[56-58] switchable surfaces[59-62] and tissue scaffolds[63-65]. Heterocycles, such as poly(pyrrole) and poly(thiophene) as well as poly(aniline) poly(phenylenevinylene), poly(p-phenylene) and various of their derivatives have been extensively investigated. The unique electronic structure of ECPs, comes from their partially delocalized π-bonds (π- π*). In their ground state they are insulating or semiconducting materials, a consequence of a phenomenon called the Peierls distortion: a one-dimensional equally spaced chain with one electron per unit is unstable and

deforms either to a zig-zag or to a periodic oscillation of spacing or twist between the units of the chain.[66, 67]

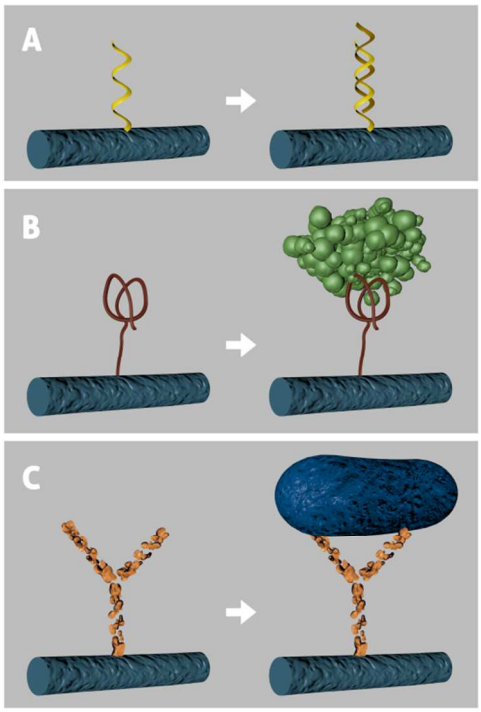

Figure 1: Schematic illustration (not to scale) of probe and target interactions in the reviewed applications. (A) Nucleic acid detection – here, single stranded probe DNA basepairs and binds its target DNA. (B) Protein detection – here, aptamer binds protein with high affinity. (C) Pathogen detection – here, antibody binds bacteria.

Upon doping (so-called based on an analogy with inorganic semiconductors) localized electronic states or self-localised excitations are created in their π- π* band gap region,[66] called positive or negative polarons, bipolarons or solitons, depending upon whether the ground state of the polymer is non-degenerate or degenerate.[32, 66] In chemical terms, doping of ECPs is a redox process that creates positively or negatively charged regions on a polymer chain – a radical cation or anion (corresponding to a positive or negative polaron) and/or a dication or dianion (corresponding to a positive or negative bipolaron).[32] Chemical[68] or electrochemical[69, 70] methods are commonly used to synthesise conjugated polymers, with electrochemical polymerizations including potentiostatic, galvanostatic and potentiodynamic modes.[36] The choice of polymerisation methodology and conditions may dictate the final properties of the polymer. Electrochemical polymerization techniques are particularly useful in biosensing applications as a direct localization of the ECP in a preferred position is possible on an electrode of any size and shape. Furthermore, the technique is simple and amenable for miniaturisation. A general approach to designing an ECP biosensor commonly employs several steps: an ECP sensing element fabrication, a recognition probe immobilization, target-probe hybridization and signal detection. A recognition probe, such as a DNA sequence or an antibody, can be immobilized on ECPs via electrochemical entrapment,[71, 72] covalent attachment[73] or affinity interactions.[74] In the mid-1980s, Umana and Waller[72] and Bartlett and Whitaker[75-77] pioneered the use of ECPs for biosensing and in particular for enzyme–based biosensing. The method provides a good control over the ECP deposition and is simple, however requires optimisation of electropolymerisation conditions to not cause any damage to the probe biomolecule.[75, 78, 79] Ghosh and Musso introduced an alternative approach of covalent attachment of DNA onto the surfaces under mild conditions.[80] The methodology is based on the use of coupling agents, such as N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and N-hydroxy succinimide (NHS), to attach the probes to a functionalised surface.[40] In the case of EDC-NHS chemistry a selective coupling between carboxylic acid- and amino- groups available on the substrate and the DNA probe respectively is facilitated. This methodology is widely utilized for a variety of biosensing designs, including proteins,[81] nucleic acids[36, 82, 83] and aptamers[84] in particular. Yet another bioprobe immobilization approach is by means of affinity interaction, commonly based on the strong affinity between avidin and biotin[85, 86] or the metal chelating coupling between nitriloacetic acid (NTA) and histidine (conveniently reversed by removal of metal ions by imidazole or EDTA).[87, 88] Following the recognition probe immobilization, the target biomolecule that specifically binds to the bioreceptor is introduced to the system.[82, 89-93] The steps of the probe immobilization and probe-target recognition both alter the electrochemical behaviour of the ECP.[36] The change in the electrochemical properties of ECPs upon the recognition event can be then measured as a change in conductivity (conductometry), electrochemical impedance (impedimetry) or potential (potentiometry). Advantages of miniaturization of ECP sensing elements in biosensing have been demonstrated, particularly in regard to achieving high sensitivities, and biosensing applications using nanowires will be reviewed here. A microarray sensing format, with possibility of sensing multiple targets is another obviously desirable outcome of miniaturisation capability. Competency in creating useful and reliable conducting polymer nanostructures is the first step towards constructing more complex devices utilising such materials, including biosensors. The following section outlines the recent developments in fabrication of ECP nanowires.

3. Fabrication of ECP Nanowires

Many well-established nanometer-scale manufacturing technologies are suitable for the fabrication of ECP nanostructures, including nanowires. These include template assisted synthesis,[94] ink-jet printing,[95] nanoimprinting,[96, 97] dip-pen lithography and related scanning probe based lithographies,[98, 99] directed electrochemical synthesis upon application of an external bias potential across metal electrodes,[100-102] e-beam lithography[91, 103, 104] and hydrodynamically focused streams.[105] Typical examples of the use of these techniques in fabrication of ECP nanowires, both

as single wires and in multiplexed formats, are highlighted in the following paragraphs.

3.1 Template Assisted Synthesis

Template assisted methods have been widely used to fabricate 1D conducting polymers as these are conceptually and experimentally simple. These have been classified into hard-template methods that use nanopore templates such as anodic aluminium oxide (AAO), or soft-template methods that use the self-assembly ability of molecules such as surfactants and DNA. Hard-template methods were pioneered by Cai and Martin in 1989.[94] ECPs such as polyaniline (PANI), polypyrrole (PPy), and polythiophene (PTh) have been synthesized inside the templates.[106-109] The first step in a template assisted method is to fill the pores of the template with monomer which can be can be achieved by simply submerging the template into a solution of the monomer, by means of a negative pressure[94], or by vapour phase deposition.[107-109] Next, the monomer is polymerised inside the template either electrochemically or using chemical oxidizing agents.[94, 106, 107-109] The last step is removal of the template and release of the formed ECP nanowires by dissolving the template in an appropriate solvent. The last step may vary greatly in difficulty depending on the nature of the template used. As examples of such a methodology, Li et al[110] copolymerized pyrrole/aniline and pyrrole/thiophene composite nanowires using AAO and Joo et al[111] studied the growth of PPy and PEDOT with different dopants and solvents in an AAO template (Figure 2A and B). Recently Guo et al[112] demonstrated an organic/inorganic P–N junction nanowire consisting of PPy and CdS, where a CdS-PPy nanowire heterojunction was produced by sequential electrochemical synthesis of CdS and PPy inside the pores of the AAO template. The nanowire displayed a strong photodependent rectifying effect (Figure 2C). The length and diameter of the nanowires synthesized using AAO templates can be controlled by polymerization time and current, and the dimensions by the template nanopores.[110, 111, 113] In addition, the electrical properties of ECPNWs can be controlled by the polymerization potentials, dopant, doping level and the nature of the template-dissolving solvents.[114] The soft-template technique is quite versatile. It is based on self-assembly of template molecules, such as surfactants or DNA, into mesophase structures.[115, 116] This technique has the advantage of a simple fabrication process, and the template removal is achieved under mild conditions or is not required, avoiding the damage that may occur during the removal of a hard template. As an example of this technique, Li et al[116] synthesized dendritic PANI nanowires by chemical oxidative polymerization in the presence of hexadecyltrimethylammonium chloride as template-forming molecule. Recently, Moon et al[117] also chemically polymerized PPy nanowires using DNA as a template on a (3-aminopropyl)triethoxysilane modified silicon wafer. Hamedi et al[44] synthesized alkoxy-sulfonate PEDOT nanowires in water using amyloid fibrils as a template.

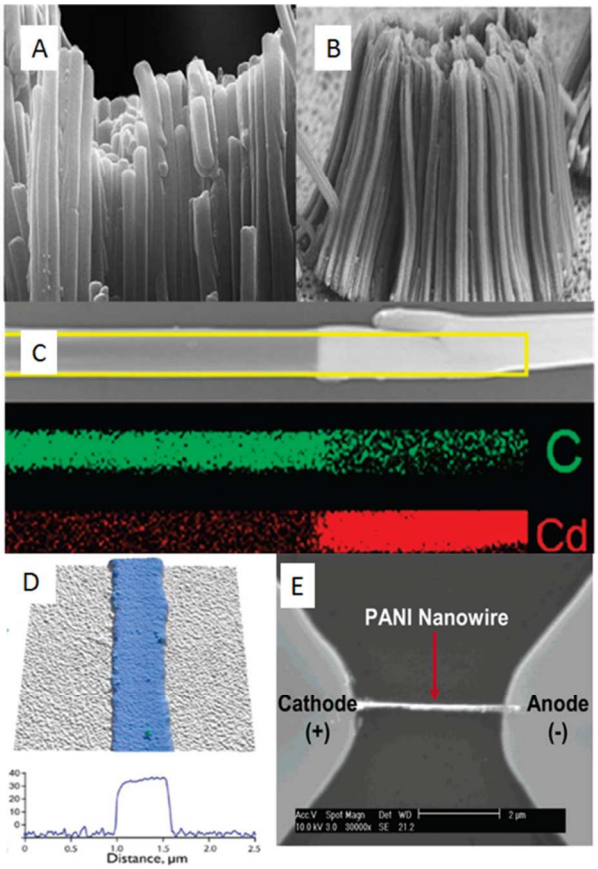

Figure 2. (A) SEM images of a PPy-CSA nanowire and (B) PEDOT-DBSA nanowire synthesized using an AAO template[111]. (C) SEM image and elemental mapping of a single CdS-PPy nanowire[112]; (D) AFM image of a 40 × 600 nm PEDOT single nanowire on glass with corresponding line scan.[118] (E) SEM image of a 100 nm width and 4 µm length PANINW.[119] A-B, Reprinted with permission from reference 111, Copyright (2003) Elsevier. C, Reprinted with permission from reference 112, Copyright (2008), and D, from reference 118, Copyright (2011), American Chemical Society. E, reprinted with permission from reference 119. Copyright (2004) American Chemical Society.

3.2 Photolithography and E-beam Lithography

Lithographically patterned templates have been used to fabricate single ECP nanowires.[104, 118, 120] For example, PEDOT nanowires have been prepared by electropolymerizing 3,4-ethylenedioxythiophene (EDOT) in aqueous $LiClO_4$ within a template prepared using so-called lithographically patterned nanowire electrodeposition (LPNE) process.[118] In such process, a conventional microfabrication method is used to produce a horizontal trench (~600 nm in width in this case) on a glass substrate, terminated by a vertical nickel electrode (Figure 2D). Immersion of such a substrate into an aqueous electrolyte solution of the monomer permitted growth of a PEDOT nanowire by oxidative electropolymerization at the nickel electrode and within the confines of the trench. Yun et al[121] used a similar technique to grow polypyrrole wires of 3 to 7 µm lengths and 200 nm in thickness between two electrodes on a silicon wafer. E-beam lithography[122] has been used to produce 1-D channels between metal electrodes covered with a resist with subsequent growth of 1-D nanostructures within the channel. Thus, Ramanathan et al fabricated single individually

addressable conducting polymer nanowires of controlled dimensions (100 nm wide and up to 13 μm long) by electrodeposition of the ECP within an e-beam patterned channel between two electrodes on the surface of a fabricated silicon wafer[71, 119](Figure 2D). This technique offers the advantages of controlling both the dimensions and the position of the NWs as the electro-polymerization occurs only in the nanochannel.

3.3 Dip-Pen Nanolithography

Dip-Pen Nanolithography (DPN)[123, 124] uses an AFM tip to deliver chemical reagents directly to nanoscopic regions of a target substrate in a controlled pattern fashion. The technique, in a tapping mode, was used by Maynor et al[125] to construct a 30 nm wide PEDOT nanowire on a conductive silicon wafer by applying a bias voltage (-12 V) between the AFM tip and the surface. The applied voltage electrochemically polymerized the EDOT monomer resulting in tip-defined deposition of PEDOT on the substrate with the width of the nanowire controlled by the applied bias voltage. Lim and Mirkin[126] reported the deposition of charged conducting polymer nanowires on an oppositely charged substrate using DPN. Electrostatic interaction between the polymer and the substrate drove the deposition of a self-doped sulfonated polyaniline (S-PANI) and doped polypyrrole as the "ink". It was possible to control the width of the polymer pattern by controlling the tip-surface contact time.

3.4 Hydrodynamic Focusing

Although DPN and e-beam lithography are attractive techniques to fabricate ECP nanowires with a diameter of several tens of nm at a desired position, these useful laboratory-scale processes are not suitable to produce large scale devices due to low yields (slow) and high costs. Fabrication methods employing microfluidics make it possible not only to synthesize nanowires in the desired position but also to produce nanowires at a low cost. In that regard, Wang et al[103] introduced a new technique to electrochemically fabricate PANI and PPy nanowires within an integrated micro-channel. The device comprised of an array of platinum (Pt) working microelectrodes, a single platinum counter electrode positioned within a microchannel and an Ag/AgCl reference electrode. The integrated microfluidic device was first filled with deionized water while a momoner solution (either aniline or pyrrole) was introduced to the electrode surface through the in-put channels. A galvanostatic step method was employed to grow the ECPNWs. The polymer nanowires were grown in the centre of the microchannels bridging the gap of the electrode junction.[103] Hou et al[105] fabricated PPy nanowires in a microfluidic system that uses characteristics of laminar flow in micro-channels. A laminar fluidic stream was used to electrochemically deposit PPy across individually addressable platinum electrode junction pairs. By using multiple microchannel inlets a focused stream was produced where the width of the stream can be altered by flow rates, and the position by the ratio of the sheath flow rates applied on both sides of the focused stream, defining a dynamic template for electrochemical deposition of PPy. The width of the PPy wire was controlled by the width of the focused stream, the gap between electrodes and the electro-polymerization time. Such techniques present an exciting approach of integrating of electropolymerization and microfluidics, offering a rapid fabrication of ECP NW and their immediate utilization in sensing applications.

3.5 Direct Electrochemical Synthesis

Direct electrochemical synthesis is a facile method to grow ECP nanowires as it is template-free, simple and does not require expensive lithographic techniques or a post-treatment for template removal. Direct electrochemical growth of single polymer nanowires between two electrodes, and without any particular prior channel fabrication other than the preparation of the electrodes, has been demonstrated[100-102, 127, 128] following the extensive research on electrochemical growth of metal wires.[129-131]

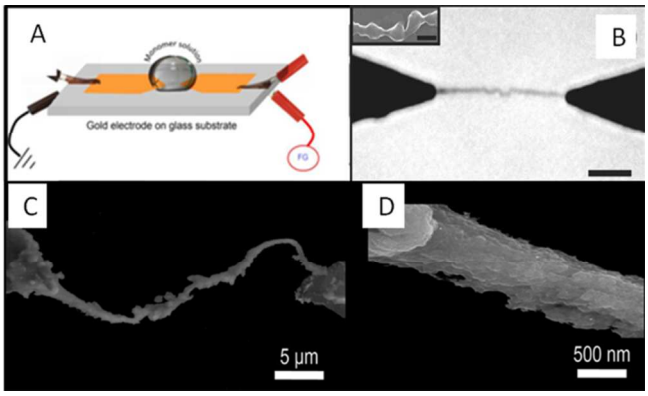

Figure 3. (A) Schematic of nanowire growth apparatus. FG designates a function generator[127]; (B) An optical microscope image of polythiophene nanowire[100]; (C) and (D) SEM images of a PEDOT: $BMIPF_6$ nanowire[42]. A, Reprinted from reference 127, Copyright (2006), with permission from Elsevier. B, reprinted with permission from reference 100, Copyright (2009), AIP Publishing LLC. C-D, Reproduced from reference 42 with permission from The Royal Society of Chemistry.

Das et al[127] electrochemically synthesized PEDOT nano-wires between Au electrodes on an insulating SiO_2 substrate (Figure 3A). A 0.1 M EDOT solution was dropped between the electrodes and a series of 100 ms duration step voltages was applied.[127] A similar technique was used by Thapa et al,[100] to grow PPy and PTh nanowires (Figure 3B). A detailed study on the electrochemical growth of PEDOT nanowires in a gap between microfabricated electrodes was also recently reported by us.[101] The work tabulates specific guidelines on the growth of PEDOT nanowires in the presence of poly(styrene sulphonic acid) (PSSA) as a dopant and shows that formation of conducting polymer wires connecting two wedge-shaped electrodes is controlled primarily by the effects of Faradaic rectification and AC-induced convection. Moreover, a highly conductive PEDOT nanowire was grown by the same approach by using an ionic liquid, 1-butyl-3-methylimidazolium hexafluorophosphate ($BMIPF_6$), as a dopant (Figure 3C and

D).[42] Raman spectroscopy mapping of the nanowires, in the presence of both dopant ions, $BMIPF_6$ and PSSA, along the length of these PEDOT nanowires, showed that the ionic liquid dopant ($BMIPF_6$) produced nanowires of highly uniform conductivity along the wires.[42]

4. Biosensing Applications of ECP Nanowires

This section is devoted to examples of where ECPNWs are utilized to sense biomolecules. The examples are grouped based on detected biomolecules focussing on oligonucleotides, proteins and pathogens. The discussions on detection of oligonucleotides and proteins are also divided based on whether single nanowires or multiple/networks of nanowires are used for sensing. There is a considerable amount of literature on ECPNW sensors that are based on the enzymatic detection of small molecules.[77, 132] As that type of sensors are strictly not detecting biomolecules they are not included in this review.

4.1 Nucleic Acid Detection

Nucleic acid analysis is greatly important in areas related to human health, such as for the diagnosis of infectious diseases and genetic mutations as well as in drug discovery, forensics and food technology. There has been an explosion of research in the last couple of decades in developing a new generation of DNA sensors with focus on detection speed, sensitivity, reliability and cost-effectiveness.[1, 133, 134] Currently, widely used gene array technologies rely on anchoring of specific probe DNA fragments or oligonucleotides onto solid surfaces and the detection of fluorescently or radioactively tagged analyte oligonucleotides that bind by Watson-Crick base pairing to the complementary probe sequence (hybridization).[134] However, gene array technology suffers from being complex and lengthy, has a limited tagging efficiency and, in some cases, hazardous waste. To address these issues novel detection approaches based on optical[135, 136], acoustic[137] and electrochemical[1, 36, 82, 89, 138] techniques have been suggested. ECPs play an important role in the electrochemical detection approaches as they have the capability to transduce a DNA recognition event directly into an electrical measurement signal[1]. One of the initial approaches to direct, electrochemical and label-free detection of DNA sequences was based on the intrinsic electroactivity of DNA, i.e. the oxidation of DNA bases[139, 140]. Using the electrochemistry of conducting polymers themselves, however, overcomes such extremes[138] and miniaturization of the ECP as nanowires further enhances the sensitivity as discussed previously. As the backbones of nucleic acids are highly negatively charged, they are expected to have a large effect on the electronic structure of the ECP to which they bind.

4.1.1 Individual ECPNWs

By positioning single ECPNWs between electrode pairs, the sensing can be done according to the principle of a field effect transistor (FET).[141] True single ECPNW FET devices were first demonstrated as a chemical sensors[119, 142] while the usability of the FET design to DNA sensing had already been demonstrated by Kim et al,[143] who utilized gold as the gate metal with the DNA coupled through thiol bonds. The two concepts of single ECPNW FET design and DNA sensing through a FET device has since been combined and refined to increase the sensitivity and lower the detection limit of the devices. In such FET devices, the ECPNW serve as a semiconductor deposited between the source (S) and drain (D) electrode of the device. When DNA is attached to the ECPNW an electric field is induced that alters the surface charge density of the nanowires and hence the S to D conductance. The target DNA can be coupled to the nanowire in different ways. An early demonstration of ECPNW DNA sensing by Ramanathan et al[71] utilized an avidin entrapped single PPy nanowire (200 nm wide) (Figure 4A) in a FET type device to detect biotin functionalized DNA.[71] First, a single PPy nanowire and avidin were electrochemically deposited between gold electrodes along a PMMA nano-channel fabricated by e-beam lithography. When a biotin-ODN probe was introduced to the system the resistance of the avidin-ECPNW increased to a constant value after 50 seconds of 1 nM biotin-DNA conjugate addition (Figure 4B) while a negligible change was observed when buffer and non-functionalized DNA was introduced. This study did not fully optimize the nanowire and thus demonstrated a rather low sensitivity, nanomolar range, but paved the way for future studies by demonstrating the response from the DNA binding event using a simple one-step incorporation of avidin (probe) and a rapid response time. The study by Ramanthan et al[71] demonstrated how the negative charge on DNA can be measured in a FET device, however it did not go further to detect the complementary binding between probe and target DNA, but rather that of avidin and biotin. A sequence specific hybridization event is what, for example, Bangar et al[144] detect by utilising a short single stranded capture probe DNA sequence. In that study, a measurement approach based on the Schottky barrier effect (SBE) was introduced in an effort to increase the sensitivity of the device (Figure 4E). The sensor efficiency was compared with the gate type FET design[144] utilizing a gating effect of ECPNW-biomolecule matrix, whereas in the SBE sensor the work function at the ECPNW-gold electrode junction[145, 146] is modulated. The PPy nanowires used in the study by Bangar et al[144] were fabricated via an aluminium template and aligned between gold patches by a magnetic field. In the above study, the probe DNA was linked either to the PPy nanowire (FET design) or to the gold electrodes (SBE design) and the introduction of the target sequence (a 19 bp breast cancer gene) to the sensor resulted in selective hybridization readout. Both sensor designs were demonstrated to be highly sensitive with the SBE design, claiming to be measuring concentrations of target DNA as low as 10^{-16} M, and the FET sensor as low as 10^{-15} M (Figure 4F). The drawback of this sensor is the difficulty to arrange the nanowires across the electrodes along with a rather complex sensor design.

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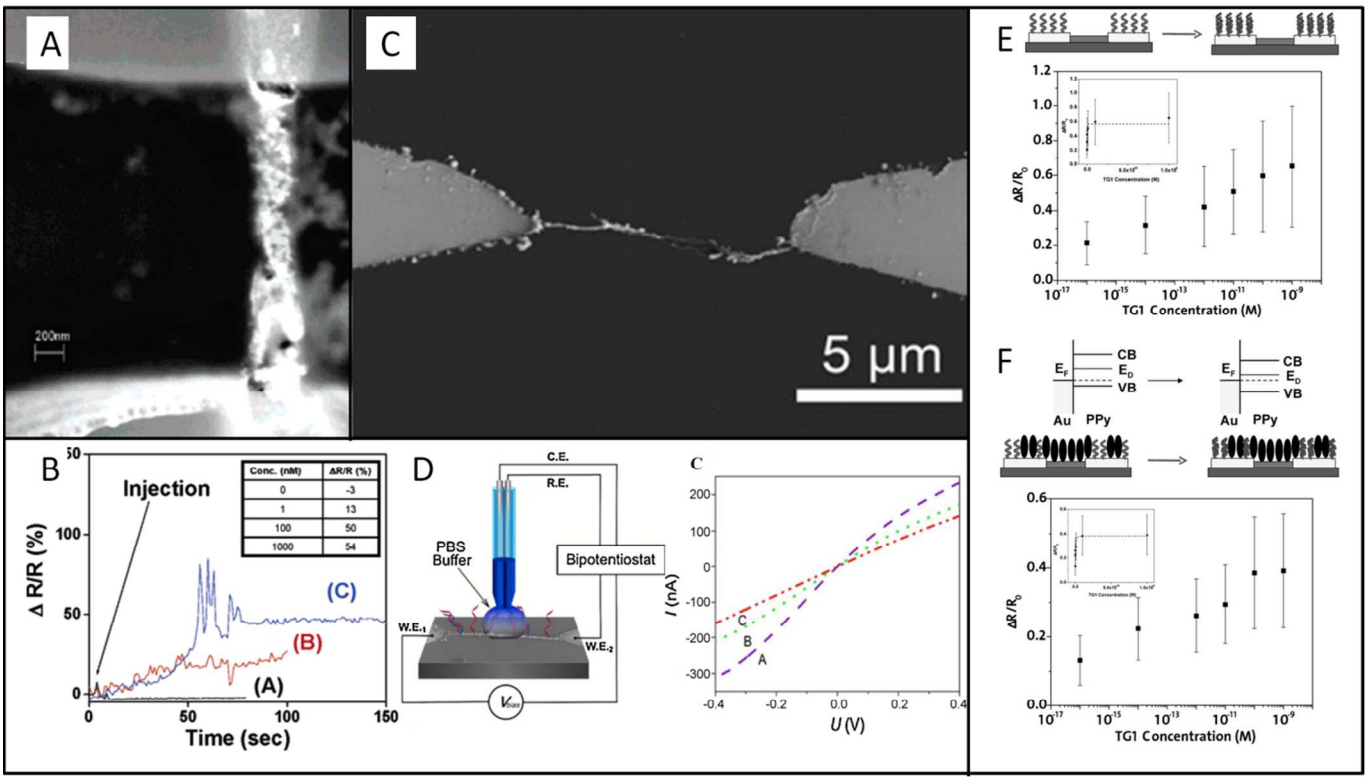

Figure 4: A) SEM image of a polypyrrole nanowire (200nm wide) embedded with avidin-conjugated quantum dots.[71] B) Electrical responses to 100nM biotin-DNA of an unmodified nanowire (A) to 100 nM biotin-DNA (single stranded) and avidin-embedded polypyrrole (200 nm) nanowires to 1 nM (B) and 100 nM (C) biotin-DNA.[71] C) ESEM image of a single PEDOT NW grown between the tips of wedge shaped gold electrodes on a glass substrate.[89] D) Experimental setup of SICM based Bio-FET (left) and overlay of I–V curves of probe-oligonucleotide modified nanowire after hybridization with increasing concentration of the complementary target oligonucleotide: (a) 0.023 pM (b) 0.5 pM and (c) 33.3 pM.[89] E) Schematic and calibration plot of the work function modulation based sensors and schematic of the energy diagram for the sensor before and after target hybridization along with F) schematic of the sensor and the calibration plot for work function based sensors with BSA blocking of nonfunctionalized surfaces.[144]. A-B Reprinted with permission from reference 71. Copyright (2005) American Chemical Society. C-D, Reprinted with permission from reference 89. Copyright (2011) American Chemical Society. E-F Reprinted from reference 144. Copyright (2010), with permission from John Wiley and Sons.

Kannan et al[73] used single PEDOT-COOH nanowires (Figure 4C) to provide a simple and practical approach for gene sensors[73] where the ECPNWs were fabricated by a template free and non-lithographic technique.[73, 101] The single ECPNWs were fabricated via electrochemical deposition of EDOT and EDOT-COOH between gold tips via applying a constant potential. The incorporation of the carboxylic acid moiety allowed for covalent coupling of the amine functionalized ODN probe to the ECPNW via well-known and highly selective EDC-NHS chemistry.[73, 82, 89] The hybridization of complementary target (sequence from breast and ovarian cancer cells), non-complementary and single mismatch DNA sequences were investigated by label-free electrical read out. The authors also, for the first time, reported a modified scanning ion conductance microscope (SICM)[147, 148] utilized to develop a three terminal FET type sensor design. *I-V* curves were recorded where two gold microelectrodes served as working electrodes and two Ag/AgCl wires in a double barrel pipette acted as counter and reference electrodes (Figure 4D).[73] Hybridization of 1 nM full complementary sequence led to 364% resistance change whereas in the case of non-complementary sequence only 6.3% difference was observed, with a detection limit claimed to be 10^{-16} M. The enhanced sensitivity and low detection limit may be attributed to the combination of three terminal electrochemistry with the FET type measurement design, which decreased the noise level of the electrical signal.[89]

4.1.2 ECPNW NETWORKS

The high surface area of PANI nanostructures[149, 150] enhances the electron transfer through the surface, making them favourable materials for solar cells,[151] biosensors,[31, 43] light

emitting devices,[152] and artificial muscles.[153] For ODN sensing, Zhang et al[43] fabricated multiple PANI nanotubes with diameter of 65-160 nm (Figure 5A) via template free self-assembly in the presence of poly(methylvinylether-alt-maleic acid) (PMVEA).[43] Successive incorporation of PANI and PMVEA provided the –COOH groups used for the binding to amine functionalized ODN probe. Both probe attachment and the following target hybridization were investigated via potential pulse amperometry by measuring the current response of pulses applied at 300-600 mV (Figure 5C).[43] The DNA attachment onto PANI nanotubes changed the surface charge density due to the accumulation of negative charges at the interface, inducing a p-type doping effect, leading to a current increase of 10 ± 0.6% (compared to 3.0 ± 0.5% change for the negative control) (Figure 5C). The detection limit of the system was determined to be moderately low at $3.4 \cdot 10^{-10}$ M. Another interesting application based on PANI was introduced by Fan et al[154] where multiple ECPNWs were utilized for micro RNAs sensing. Micro RNAs (miRNAs) are small non-coding RNAs that take part in the regulation of gene expression by binding to messenger RNA hindering translation or leading to messenger RNA degradation.[155] miRNAs have also been implemented in human cancer[156] which makes them an important biosensing target. In the study by Fan et al,[154] the read-out in fact comes from the enzyme catalysed polymerization of aniline monomers upon their electrostatic interaction with miRNA (serving as the charge provider for the oxidation of aniline) captured by peptide nucleic acid (PNA)[157] immobilized in nanogaps between interdigitated microelectrodes. As the monomers aligned towards the negatively charged RNA strands, the PANI formed onto the hybridized target miRNA, with resultant formation of a nanowire network with a conductance directly correlated to the amount of hybridized target. The detection limit was found to be $5 \cdot 10^{-15}$ M and the specificity over a single base mismatch sequence was excellent.[154] The use of PNA as the capture probe is also noteworthy. PNA is more stable than RNA or DNA, has a higher binding strength to complementary sequences and a better specificity.[157] Although these properties of PNA are useful for any biosensor, in the present case, the non-acid nature of PNA is the most significant, as this discriminates between probe and target and reduces unwanted adsorption of aniline monomer to the probe. The concept of using the enzymatic polymerization of aniline as the read-out has been further developed in a recent paper by Hao et al[158] where the aniline monomer is covalently attached to a self-assembling peptide. The aniline conjugated peptide readily self-assembles into thin, uniform amyloid-like nanowires subsequently functionalized with a hairpin loop probe DNA. When the target (Hepatitis B gene) binds the probe, the hairpin opens up and allows the binding of a third sequence which is conjugated to the enzyme horse radish peroxidase, which in turn polymerizes the aniline monomers leading to a large increase in conductivity.[158] The developed sensor showed very low detection limit (10^{-15} M) and was able to discriminate target from single nucleotide mismatch sequences.

The incorporation of metal particles into ECP nanomaterial may enhance the sensitivity of the device. Feng et al[159] employed this property in their study of Au-PANI nanotube membranes where they combined the large surface area of nano-PANI, high conductivity of Au nanoparticles and the film-forming properties of chitosan. This study also compared two different probe DNA immobilization methods, namely adsorption through immersion and electro-deposition of DNA at constant potential. Impedance studies revealed that immersion adsorption was more efficient than electro-deposition. Hybridization of target DNA was performed by applying 0.5 V constant potential during 500 seconds. Detection of hybridization was done by impedance spectroscopy and the detection limit was reported to be 10^{-12} M.

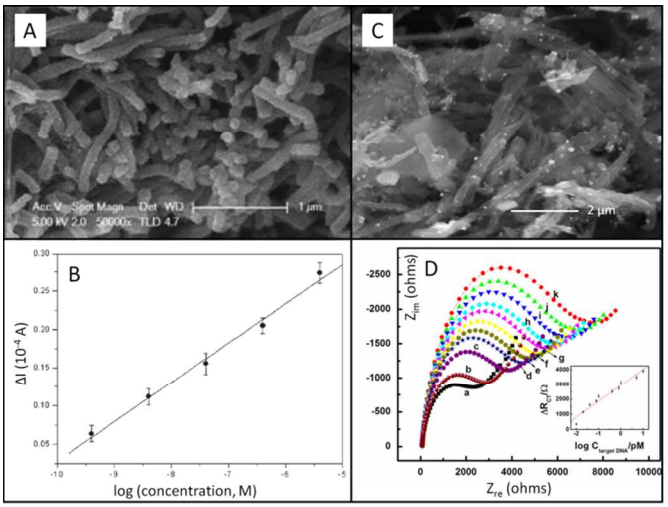

Figure 5: SEM images of A) PANI[43], B) PPy-PEDOT-Ag multiple nanotubes.[160] C) Current change versus target DNA concentration of PANI nanotube sensor[43], D) EIS measurements of PPy-PEDOT-Ag-S-ssDNA in the presence of (a)0, (b)1.0 10^{-14}, (c) 2.0 10^{-14}, (d)4.0 10^{-14}, (e)8.0 10^{-14}, (f)1.0 10^{-13}, (g)4.0 10^{-13}, (h) 8.0 10^{-13}, (i)1.0 10^{-12}, (j)5.0 10^{-12} and (k)1.0 10^{-11} M target DNA concentration. Inset: variation of ΔR_{CT} with log (C_{target} DNA)[160]. Panel A and C reprinted with permission from reference 43. Copyright (2007) John Wiley and Sons. Panel B and D Reprinted from reference 160, Copyright (2013), with permission from Elsevier.

As an alternative to PANI nanotubes, Radhakrishnan et al[160] fabricated PPy-PEDOT-Ag nanotube composites (Figure 5B). The conducting polymer part of the nano composite provided large surface area while the Ag nanoparticles enhanced the conductivity and served as an anchor for DNA immobilization, thereby removing the need to use functionalized CPs. Both probe immobilization and target hybridization were detected via electrical impedance spectroscopy (EIS) measurements, where the results were fitted into equivalent circuit diagrams to calculate the charge transfer resistance. A small charge transfer resistance was observed for the PPy-PEDOT-Ag nano composite (31 Ω) which increased to 2248 Ω after probe attachment and followed by further increase to 6181 Ω after target hybridization. The detection limit was determined to be $5 \cdot 10^{-15}$ M (Figure 5D), which is indeed excellent for this type of sensor design. Previous studies utilizing similar systems have published detection limits of 10^{-12} M ((PANI-Au) nanocomposite)[161] and 10^{-13} M ((PPy–PANi–Au) nano

composite film).[162] A recent study utilized a nanocomposite of PPy and PANI without added metal particles for the label free detection of DNA[163] and achieved a detection limit of $5 \cdot 10^{-14}$ M. The comparatively low detection limit was proposed to be an effect of the nanoproperties of the polymer and the charge storage properties of the PPy-PANI composite. In addition, methylene blue was used as a DNA intercalator to enhance the signal. The study show particular merit in the simple fabrication and modification protocol used, utilizing glutaraldehyde as a linker for the probe DNA binding.[163]

4.2 Protein Detection

The detection of proteins has also attained a burgeoning interest in last decade as it enables sensitive diagnosis of genetic abnormities and diseases. In particular, the selective and sensitive detection of cancer markers is vital for cancer monitoring, early diagnosis and for defining the disease state.[164] Several different materials (and detection mechanisms) have been used to construct protein biosensors, such as carbon nanotubes,[165, 166] conducting polymer films[167] and silicon nanowires.[168] Functionalization of ECPNWs with selective probes such as antibodies,[169] avidin[170] or aptamers[171] have enabled them to be exploited as protein sensors. Amongst the various possibilities, aptamers have emerged as most promising probes for protein recognition, as they are 1) highly specific to their targets,[172, 173] 2) relatively cost effective and chemically robust compared to antibodies[173, 174] (especially when based on PNA[157, 175]) and 3) possible to prepare artificially and thereby easily engineered for specific purposes.[176, 177] Furthermore, the smaller size of aptamers as compared to antibodies brings the sensing event closer to the ECPNW interface, reducing the effect of charge screening and generally increasing the sensitivity.[45, 93, 174] Incorporation of aptamers with ECPNWs can be carried out via entrapment by in-situ polymerization[90] and/or by covalent attachment, commonly to carboxylic acid groups of functionalized CPs.[91, 174] Both the immobilization of the aptamer and the specific protein binding can be detected via FET type electrochemical measurements.[90, 91, 174, 178].

4.2.1 Individual ECPNWs

Huang et al[90] electrochemically deposited PPy single nanowires with entrapped aptamers along poly(methylmethacrylate) (PMMA) nano channels between the gold electrodes by applying constant current to solution containing monomer, aptamer and NaCl. The incorporation of fluorescently labelled aptamer confirmed the successful fabrication of nanowires with entrapped aptamer (Figure 6A). The study also presents sensing results using aptamers towards Immunoglobin E (IgE) (as proof of concept) and Mucin 1 (a well-known human cancer marker overexpressed in a range of epithelial tumors). The selective binding of IgE led to an increase of the conductance of the sensor, which was attributed to the net negative charge of IgE at the pH used (7.4), leading to an increase of the negative charge density of the ECPNW.[90] Further additions of IgE resulted in a stepwise increase of conductance whereas the negative control remained constant (Figure 6B) and a detection limit of 10^{-11}M was achieved. The sensor incorporating a Mucin 1 aptamer also responded with an increase in conductivity upon binding of the negatively charged cancer biomarker. The detection limit for Mucin 1 was determined to be slightly higher ($2 \cdot 10^{-9}$ M) but still significantly better than the clinically used ELISA assay.[90]
CA 125 (or Mucin 16) is another well studied cancer marker, primarily related to the diagnosis of ovarian cancer.[179] A ECPNW biosensor for CA 125 was developed by Bangar et al,[92] where the nanowires were fabricated in a template directed manner and aligned/manipulated to connect to gold microelectrodes. The authors studied both NHS-EDC and glutaraldehyde chemistries for the functionalization of nanowires with antibodies relevant to the CA 125 and concluded that in EDC chemistry was more efficient.[92] The sensing of the antigen was performed in spiked human blood plasma or 10 mM phosphate buffer (Figure 6C). A detection limit below 1 U/ml (no correlation between U and mass or molar concentration given) and a broad dynamic range was detected via FET type conductance measurements (in both buffer and spiked human blood plasma), which indicates the applicability of the sensor for clinical use. In this case, the response for the binding of this protein was a decrease of the conductivity of the nanowire (Figure 6D-E) but the charge of the protein was not stated.[92] Similar functionalization and characterization methods were employed for a PANI single nanowire sensor developed by Lee at al.[180] In this case the nanowires were electropolymerized in a nanochannel between two electrodes. The PANI wires were functionalized with antibodies towards either IgG (proof of concept) or myoglobin (a cardiac biomarker). The detection was done by measuring the conductance of the nanowire in a FET type design. The binding of IgG (net negatively charged) gave rise to an increase in conductance, and a detection limit of 3 ng/ml was achieved. After optimization of antibody density etc. using IgG, myoglobin was also successfully detected using a myoglobin antibody. Myoglobin also carries a net negative charge and gave rise to an increase in current upon binding and a low detection limit of 1.4 ng/ml, well within the biologically relevant concentration range.[180] Another example of utilizing PPy nanowires for the cancer biomarker recognition is the electrochemical detection of Vascular Endothelial Growth Factor (VEGF).[93] First multiple nanowires of Py and pyrrole-2-carboxylic acid (P3CA) were obtained by chemical polymerization in a reverse emulsion system and manipulated onto interdigitated FET type micro electrodes (Figure 7 A-C) (where the ECPNW was WE, Ag/AgCl was RE and Pt wire was CE). In this study, a RNA aptamer was utilized for the selective binding of VEGF and characterized by real time, label free, three terminal electrochemical measurements. The binding of the positively charged VEGF led to a decrease in the current response (recorded at a constant potential of -50 mV) of the sensor (Figure 7D) and a detection limit of $4 \cdot 10^{-13}$ M was reported for the most successful aptamer.[93] An example of multiple, but parallel and aligned ECPNWs, PEDOT-COOH was used by Xie et al[174] for the label free detection of thrombin.

There are well studied aptamer sequences for thrombin, which makes thrombin a suitable target to study along with its important enzymatic function of converting fibrinogen to fibrin in blood.

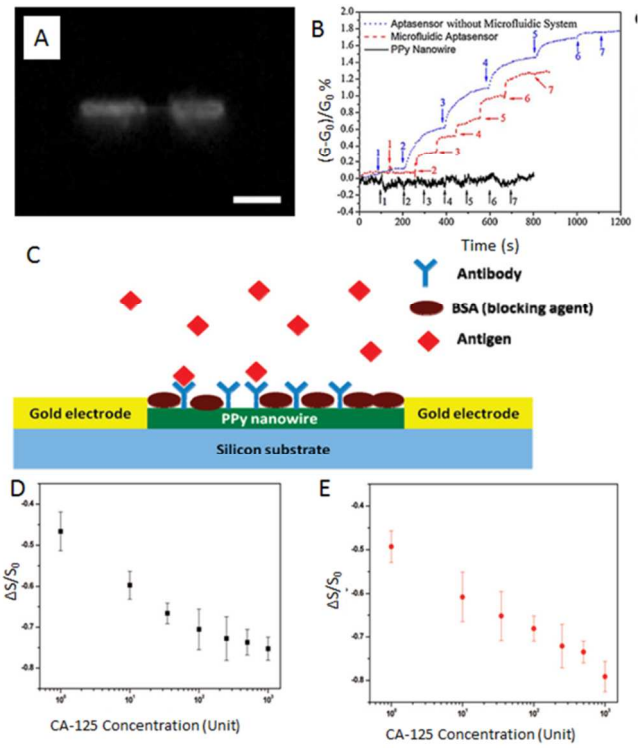

Figure 6: A) Fluorescent microscope picture of PPyNW revealing the successive attachment of aptamer and nanowire.[90] B) Real-time responses of aptasensor and PPy nanowire in the presence of (2–6) 0.1, 1, 10, 100, 1000 nM IgE and the response of microfluidic aptasensor in the presence of (2–6) 0.01, 0.1, 1, 10, 100 nM IgE.[90] C) Selective attachment of antigens (CA125 cancer biomarker) to the antibodies immobilized onto PPy NW.[92] Conductivity change of nanowire as a result of target hybridization in D) PBS[92] E) spiked human blood plasma[92]. A and B Reprinted from reference 90, Copyright (2011), with permission from Elsevier. C-E, Reprinted with permission from reference 92. Copyright (2009) American Chemical Society.

The nanowires in the study by Xie at al[174] were fabricated via template free direct electrochemical growth of EDOT-COOH monomer between Au electrodes. After electrochemical growth, amine modified thrombin-binding aptamer was covalently attached to the –COOH carrying nanowire via NHS-EDC chemistry. Both probe and target protein immobilizations were monitored via label free conductance measurements based on FET type design. Following the attachment, negatively charged aptamer increased accumulation of negative charges onto the ECPNWs and hence the source-drain current. The binding of thrombin (net positive charge at pH 5) resulted in a decrease of the current, attributed to the neutralization of negative charges on the surface. The detection limit was in the range of 10^{-9} M, with a dynamic range covering physiologically relevant concentrations.[174] Optimization of nanowire fabrication and complex responses of probe attachment / target binding events are the main challenges of this study.

Not only aptamers and antibodies can be used to bind and detect protein. In studies by Arter et al,[104, 181] viruses were incorporated into PEDOT nanowires to enable binding to and sensing of various proteins. The virus chosen was the M13 bacteriophage, which can display surface receptors for a range of molecules, proteins or DNA. The receptors on the surface are selected from phage display libraries developed through in vitro selection. Therefore, by incorporating the M13 bacteriophage into PEDOT ECPNW sensors, a truly versatile sensor is created. Linear arrays of M13-PEDOT hybrid nanowires were produced by lithographically patterned electrodeposition[104] and in an initial study M13 antibodies were used to demonstrate the scope of the sensor, where the binding of the antibody led to an increase in the resistance of the ECPNWs.[104] More importantly, the work was followed up by including a M13 phage selected for displaying receptors for prostate specific membrane antigen, an important prostate cancer marker.[181] The sensor responded with a concentration dependent increase of the resistance upon binding of the net negatively charged prostate marker with a detection limit of $6 \cdot 10^{-8}$ M. The larger size of the incorporated virus as compared to aptamers used in other sensors[90, 93, 174] may contribute to the higher detection limit, which is currently insufficient for clinical use.[181]

4.2.2 ECPNW NETWORKS

With their simpler fabrication, networks of ECPNWs are a viable alternative for the detection of protein. Yoon et al[91] fabricated multiple nano tubes via copolymerization of pyrrole-3-carboyxlic acid (P3CA) with pyrrole (PPy-P3CA) in a reverse micelle emulsion system (Figure 7E). The fabricated sensor was utilized for aptamer mediated detection of thrombin. The nanowires were deposited on, and covalently bound to, interdigitated electrodes and the DNA aptamer was designed with an amino linker facilitating its binding to the carboxylic groups present on the ECPNWs (Figure 7F).[91] The FET type measurement detected a current decrease upon binding of the positively charged thrombin to the aptamer, in agreement with the study by Xie et al on single ECPNWs.[174] The detection limit was determined to be $5 \cdot 10^{-8}$ M,[91] not quite as good as that reported on the single nanowires.[174] A rather different approach, based on the use of synthesised nitriloacetic acid (NTA) pyrrole,[182] circumvents the need for use of an aptamer or an antibody. Instead the binding of histidine-tagged proteins to chelated bivalent cations on the NTA can be measured directly.[178] This is useful for the detection and quantification of cloned gene products. Here, poly(pyrrole)-nitriloacetic acid PPy-NTA nano tubes were obtained via electropolymerization through an alumina template, aligned along the gold electrodes and functionalized by Cu^{+2} ions[178] and used to recognize the histidine-tagged Syntaxin protein (His5-Syntaxin). A FET type design was used and *I-V* curves obtained via source-drain responses were used for label free monitoring of protein binding. While the binding of Cu^{2+} led to a decrease of the resistance, the binding of the protein was detected as a resistance increase. A low detection limit of 10^{-14} M was

achieved for the detection of Cu^{2+} and the sensor was shown to respond to a concentration of 1 ng/ml of Syntaxin.[178]

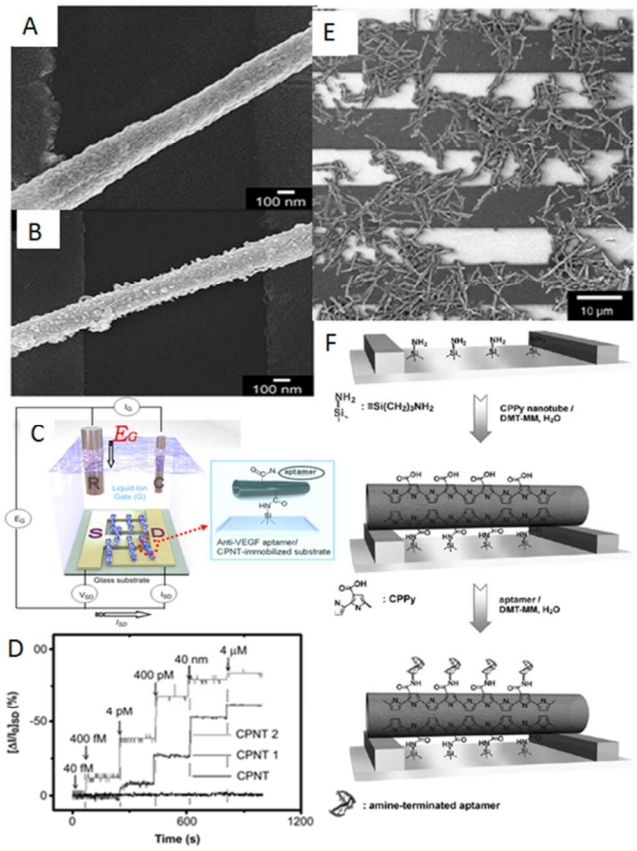

Figure 7: FE-SEM images of A) before[93] and B) after immobilization of aptamer onto ECPNW[93]. C) Working mechanism of three terminal electrochemistry and FET combination[93]. D) Current measurements of ECPNW after target hybridization with different concentrations.[93] E) FE-SEM image of PPy nanowires deposited interdigitated electrodes.[91] F) Schematic representation of NW-Au surface and NW-amine terminated aptamer attachment with DMT-MM chemistry.[91] A-D, reprinted from reference 93, Copyright (2010), with permission from Elsevier. E-F, reprinted from reference 91, Copyright (2008) with permission from John Wiley and Sons.

4.3 Pathogen Detection

The detection and identification of viruses is crucial to prevent fast spread of contiguous diseases and also for the early diagnosis of infections to start the appropriate treatment. The conventionally used biological assays for viruses detection suffer from taking a long time to produce results, or lack of sensitivity to contaminants or use of labels.[183-185] These methods, such as ELISA, amplification of viral nucleic acids with PCR and immunofluorescence, fail in field as they require complex instrumentation and fully-equipped laboratories.[186] It is of practical importance to develop cost effective, rapid, portable, sensitive and selective virus biosensors allowing 'on spot' detection, now enabled through progress in nano technology and bioengineering. To date, CNTs,[187] silicon nanowires,[188] magnetic nano particles[189] and ECPs[185, 186] have been utilized in electrochemical virus sensors. The examples of ECPNWs for whole pathogen detection are still rather limited. Shirale et al[185] fabricated PPy nanowires via template directed electrodeposition using a 200 nm pore size alumina membrane and employed them for label free detection of viruses (bacteriophages T7 and MS2).[185] To produce a FET type sensor the ECPNWs were then aligned by AC dielectrophoresis to span the gaps between fabricated gold microelectrodes, followed by manual removal of excess nanowires using a fine gold probe.[185] The ECPNWs were subsequently functionalized with (negatively charged) antibodies using NHS-EDC chemistry and verified by AFM and *I-V* curves as an increase in both diameter and the resistance of the nanowire. Both developed sensors showed a detection limit of 10^{-3} Plaque Forming Unit (PFU) in both buffer and spiked lake water (detection limit should be given as PFU/volume, but volume was not specified). The authors acknowledged that the sensor responds to all virus particles, not only those infective, which means that the concentration measured may be a lot higher (up to a million times higher) than what the Plaque Forming Unit assay indicates.[185] Both sensors were found to be selective towards their targets, however with a better selectivity achieved with the monoclonal anti-MS2 antibody as compared to the polyclonal anti-T7 antibody.[185] Another example of ECPNWs for virus detection is that by Chartuprayoon et al,[186] where PPy nanoribbons were conjugated with polyclonal antibodies towards the cucumber mosaic virus (CMV). The authors carefully investigated the effect of factors such as the diameter of the nanowires, their conductivity and the ionic strength on the sensitivity of the sensor. The fabrication of the nanoribbons was performed via lithographically patterned nanowire electrodeposition,[190] a combination of e-beam photolithography and electrochemical polymerization. PPy nano ribbons with 1 cm length, 500 nm width and thicknesses from 25-100 nm were obtained along the nickel (Ni) nano bands. The detection of virus was performed via I-V curves obtained with FET type measurements with a good dynamic range from 10 ng/ml to 100 µg/ml. Both immobilization of antibody and hybridization of target virus led to the increase of the resistance of the ECPNWs. It was also found that lowering the conductivity as well as decreasing the thickness of the nanoribbon both increased the sensitivity of the sensor. Lowering the salt concentration of the sensing solution (thus increasing the Debye length) was also identified as leading to better sensitivity, but at the expense of sample to sample variation.[186] In the study by Chu Van et al[191], a biosensor towards Japanese Encephalitis Virus was developed based on the immobilization of polyclonal antibodies onto a network of PANI nanowires on interdigitated Pt electrodes. The detection was performed using impedance spectroscopy and the limit of detection was found to be less than 10 ng/ml.[191] A few cases of ECPNW sensors for bacteria detection have also been reported.[192-194] The studies by Langer et al are based on the non-specific interactions of bacterial cells with the conducting polymer fibers,[193, 194] while the study by Garcia-Aljaro et al[192] utilized the immobilization of a monoclonal antibody onto PPy nanowires for the specific detection of B. globigii spores, as a model for bio warfare agent B. anthracis spores. The single nanowires were assembled onto interdigitated electrodes and the change in resistance of the

ECPNWs was measured in using a FET type design. A good detection limit (1 colony forming unit/ml) was reported.[192]

5. Transduction principles

The response mechanisms of chemically sensitive semiconductors have been intensively studied for some time.[195, 196] One of the main advantages of using ECPs for biosensors is their ability to act both as the sensing element and the transducer of the signal. As a high-level concept, the transduction mechanism seems simple: the binding of, particularly charged, molecules alters the electronic structure and charge distribution near the surface of the material and thus the electrical resistance. By confining the current path in the material to be close to the surface, simply by confining the material itself, the response from a binding event can be greatly enhanced. If the sensed medium is a gas, this simple, high-level interpretation can be developed successfully.[197] However, when the sensed medium is a fluid, the possible effects that give rise to a signal multiply and possible artefacts abound.[198] The type of binding molecule, the type of ECP used and the doping state of the polymer (which will depend both on the initial preparation and the subsequent history) all matter for what signal is transduced by the ECPNW. For simple small molecules for example, the diffusion of the molecule into the polymer can lead to a reaction with the ECP, changing its doping state.[132] For an ammonia sensor based on PANI nanowires, the exposure to ammonia led to rapid dedoping of the PANI resulting in a decrease of charge-carrier density and a reduced conductivity.[199] The response time was seen to be dependent on the diameter of the nanowire, due to its effect on the diffusion time of the gas into the wires.[199] For larger and more complex molecules, the transduction principles become less clear cut. It is clear that there are large signals consequent upon biomolecule binding to an ECP interface, and that the use of ECP nanowires can give devices of exquisite sensitivity. However, practical implementation will require that the different influences upon the signal are understood and controlled.[198] Some of the possible effects can be listed:

- Binding of a charged biomolecule changes the electric potential distribution near to the interface both in the solution and in the polymer. The ionic composition of the solution adjacent to the polymer interface is changed as a result. There is a significant effect on the impedance of the polymer-solution interface[200, 201] which will change the electrical coupling to the solution and hence the measured impedance of an immersed nanowire.[198]
- Ion exchange across the polymer-solution interface may change the dopant concentration within the polymer near to the interface. The charge carrier concentration near the interface is dependent on the concentration of both fixed and mobile dopants and on the electric field induced by the surface charge of bound species.[196]
- Charge carriers near the interface may be trapped by local deformations of the polymer at the interface. The local deformations and hence the concentration of these trapping states will depend on the electric field across the interface and hence on the surface charge of adsorbed and bound species and the distribution in space away from the surface.
- The electric field across the interface, which drives both the local distortions of the polymer and the exchange reactions of mobile ions, is dependent on the electrical double layer at the solution side of the interface and hence on the electrolyte ionic strength as well as on the spatial distribution of bound charges in the interface region.[202] Changes in the electric field consequent on changes in binding of molecules to the interface depend both on the magnitude of the charge change and on the charge distribution away from the interface.
- Ions at the interface may both be absorbed into the polymer surface and adsorbed onto it. These effects will be dependent on what other species are adsorbed or bound and by changes in these. Ionic species interact with the charge carriers, whose mobility and concentration will change as a result.

If the nanowire resistance measurement is made in air after target binding then rinsing and removal of the solution,[73] then the effects of the solution on the measured resistance are removed although the charge distribution near the interface will be perturbed by the drying and dependent upon the details of the history of the system. In this particular example, the interpretation of the effect was framed in terms of effects of the surface charge on trapping of carriers at the interface.[73] The charge carriers in the conductive polymer are in the form of positive charges on the polymer backbone which cause a local deformation of the polymer and which interact with ionic species trapped within the polymer. If a strongly negatively charged molecule such as DNA is bound to the surface then a part of the counter charge to surface-bound oligonucleotide will be in the form of positive polarons at the surface of the polymer even after removal from the solution, rinsing and drying. These charges could then become trapped by the electrostatic field associated with the surface-bound molecules. The consequent local deformation of the polymer backbone would result in the formation of trapping states for charge carriers leading to an increase in resistance of the nanowire.[73]

The complexity of the transduction of binding into conductivity change that is implied by the discussion above is evident in the literature. For measurements made in solution, literature reports do not even agree on the sign of the effect for a given type of target molecule, particularly when the target is a protein. Importantly, different proteins can have different net charge, a detail that is not always stated in the studies.[92, 178] Furthermore the charge of the capturing molecule (aptamer or antibody) and its conformation before and after binding of the target is also likely to affect the response seen. It is also worth noting that the net charge of a protein is small compared to that

of DNA and that subunits or regions of the protein may have the opposite charge to the overall net charge, so the orientation and conformation of the captured protein may also affect the outcome, along with the local pH and ionic strength. From the publications reviewed in the sections above though a trend emerges: when an aptamer is the probe, the subsequent binding of positively charged proteins led to an increase in resistance,[91, 93, 174] the opposite effect as can be expected from the DNA cases. This has been explained as being due to a decrease in mobility of charge carriers as the negative charge of the aptamer is screened, which contradicts explanations made for DNA sensors[73] and may instead be due to the conformational changes the binding event causes on the aptamer – collapsing negative charges closer to the surface of the nanowire. This argument however does not fit with observations by Xie et al,[174] who studied the effect of the binding of the aptamer to the ECPNW and observed a decrease in the resistance (it is worth noting though, that this study does not use any supporting electrolyte during the nanowire fabrication).When binding of negatively charged protein was studied a decrease of the resistance was found when the capture probe was an aptamer[90] or an antibody,[180] while an increase in the nanowire resistance was found when the capturing agent was a virus.[181] Clearly, more work and careful analysis of transduction principles will be beneficial for the development of future sensors.

6. Summary and Outlook

ECPNWs provide a versatile route towards sensors for biomolecules. The unique blend of properties of these materials, such as the intrinsic conductivity of the polymers used, their simple functionalization, and the sensitivity imposed through their one dimensionality provides a framework for the development of a range of sensors. Compared to other nanomaterials, the fabrication of ECPNWs is effective and simple. There is a range of methods for the fabrication of ECPNWs, with perhaps the most promising ones being those where the nanowire can be polymerized in-situ, when and where needed, through direct electrochemical synthesis.[42, 100, 101, 127] The abundance of material that can be made available through template assisted synthesis also widens the scope of the type of sensors that can be constructed, and template assisted methods are available that do not require subsequent template removal[115, 117, 203]. The ECPNWs can be functionalized with biomolecules after the fabrication, either by using protocols available for the standard CP monomers, pyrrole in particular, or by incorporating modified CP monomers in the synthesis. The specific biomolecule binding can be detected in a label-free manner by exploiting the environmental sensitivity of the conductivity of the ECPNWs. The readout is electrical, with methods such as EIS or resistivity being commonly used. The signal transduction arises from effects on the charge mobility of the ECP from the binding events, or from effects on polymer conformation. Although the signal transduction is rather well understood for simple biomolecules, such as DNA, more work is needed to fully understand the signals arising from protein or pathogen binding. The sensitivity and selectivity achieved with the ECPNWs is often very good and competes well with other types of sensors. The miniaturization also brings other advantages, such as only needing very small sample volumes and providing the possibility of arraying formats, by for example microspotting different probe DNAs onto an array of individually addressable ECPNWs. A new generation of biosensor devices can be expected to integrate on-chip microfluidics with an array of parallel sensors with a panel of probes relevant for example for a particular disease or risk profile. Challenges of ECPNWs have been their environmental stability and the consistency of fabrication methods, areas where progress is continuing to be made.

Acknowledgements

Funding from the University of Auckland, The MacDiarmid Institute for Advanced Materials and Nanotechnology and Auckland UniServices Limited is gratefully acknowledged.

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