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Cite this: *Lab Chip*, 2012, **12**, 2612–2620

www.rsc.org/loc

CRITICAL REVIEW

Recent progress in the design of nanofiber-based biosensing devices

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Received 13th December 2011, Accepted 5th April 2012
DOI: 10.1039/c2lc21240d

This review addresses recent progress made in the use of nanofibers for analyte detection and sample preparation within analytical devices. The unique characteristics of nanofibers make them ideal for incorporation within sensors designed to allow for sensitive detection of clinical, environmental, and food safety analytes. In particular, the extremely large surface area provided by nanofiber mats and arrays drastically increases the availability of immobilization sites within biosensors. Additionally, nanofibers can be made from a variety of biocompatible materials and can be functionalized through the incorporation of nanoscale materials within spinning dopes or polymerization solutions. Finally, methods of nanofiber formation are largely well understood, allowing for controlled synthesis of nanofiber mats with specific sizes, shapes, pore sizes, and tensile strengths. In this paper, we present a survey of the different materials that are currently being used to produce nanofibers for use within sensing devices. In addition, we compare the limits of detection and linear ranges of nanofiber-based sensors and conventional sensors to determine if detection is improved by the inclusion of nanoscale materials.

Introduction

Materials with dimensions on the nanoscale (nanomaterials) are increasingly being integrated within analytical systems to allow for the detection of low concentrations of analytes without complicated amplification processes such as polymerase chain reaction (PCR) and nucleic acid sequence base amplification (NASBA).[1–3] One of the main advantages of nanomaterials is their extremely high surface area to volume ratio, which increases the number of binding sites available for biological recognition element immobilization. In addition, the use of nanomaterials can result in faster mass transfer rates, resulting in lower limits of detection and faster analyte detection rates than those seen in conventional sensors.[2] Nanofibers, nanowires, and nanotubes are frequently investigated for use within biosensors. The main distinction between nanofibers and nanowires lies within their sizes. Nanowires generally have diameters on the order of 10s of nanometers,[4,5] while nanofibers are typically defined as having diameters less than one micrometer.[6,7] Nanotubes and nanofibers differ primarily in their structures. Nanotubes typically consist of hollow cylinders, while nanofibers can have a variety of different structures and are normally solid.[1,8,9] In particular, carbon nanotubes and carbon nanofibers have distinctly different structures. Carbon nanotubes are composed of concentric hollow graphene cylinders,[8] while carbon nanofibers are composed of graphene layers that form stacked cones, cups or plates.[1] Several groups have demonstrated the successful fabrication of sensitive biosensors using one-dimensional nanostructures such as carbon nanotubes and single nanowires.[10–12] These sensors utilize the fast mass transfer and large surface areas provided by the nanomaterials. However, single nanowire sensors can exhibit high background noise and variable signals.[2] In addition, the reproducible synthesis of carbon nanotubes and single wires is often difficult and many fabrication processes have poor control over the size, shape and densities of the materials produced.[1,10] Consequently, many nanomaterial-based biosensors have variable signals, making them ill-suited for commercialization. In order to address these limitations, non-woven nanofiber mats and arrays are being examined as alternatives to one-dimensional nanostructures.[2] An advantage of nanofiber mats and arrays is that their entire surface area can easily be functionalized with nanoscale materials.[1] Carbon nanofibers have oxygen-containing active sites along their surfaces, which facilitate functionalization.[1] Nanofibers composed of other materials, such as polyaniline and chitosan, can also be fabricated with different chemical groups on their surfaces, such as sulfonic acid[13] and amino groups.[14] Electrospun nanofibers made of various materials can be further functionalized through incorporation of nanoscale additives in the polymer spinning dopes. On the other hand, carbon nanotubes have a closed shell structure that limits how they can be functionalized.[1] The structure of a perfect single walled carbon nanotube is without functional groups and consequently relatively chemically inert.[15,16] While several functionalization methods have been described, they are often complicated and require the fibers to undergo chemical treatments after synthesis.[15–17]

Nanofibers can be produced by a variety of methods, including electrospinning, interfacial polymerization, catalytic synthesis, dilute polymerization, and chemical vapour deposition.[18]

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These processes are generally well-understood and allow for controlled synthesis of nanofibers with specific sizes, shapes, tensile strengths, and chemical functionalities.[18,19] Nanofibers can also be made out of several materials that exhibit high chemical stability and biocompatibility, allowing them to be used in a variety of conditions and with a variety of analytes.[18–20]

Electrospinning is a nanofiber synthesis method that has been used for over 75 years.[21] During electrospinning, electrical forces are used to form ultrathin fibers from polymer spinning dopes.[18,19] The fibers formed during electrospinning have diameters on the order of 100 nm, though smaller fibers can be produced.[19,22] A typical electrospinning apparatus consists of a spinneret (typically a syringe) containing the polymer spinning dope, a pump, a high voltage source, and a grounded collector plate (Fig. 1). During electrospinning, the pump is used to slowly push the polymer solution out of the spinneret. The tip of the spinneret is attached to a high voltage power source in order to confer a constant charge on the polymer solution. When subjected to an electrical force, the polymer solution will form a cone, called a Taylor cone, at the tip of the spinneret.[23,24] A grounded collector plate is placed across from the spinneret, and the polymer solution accelerates towards the collector plate when the electrostatic forces between the collector plate and the spinneret overcome the surface tension at the spinneret tip.[18] After leaving the spinneret, the polymer solution undergoes whipping, and the solvent evaporates, resulting in a solid polymer fiber.[19] The nanofibers accumulate on the collector plate, forming non-woven mats with extremely high surface area to volume ratios and small pore sizes (Fig. 2).[23]

Parameters that affect the spinnability of a polymer melt include spinning solution concentration and viscosity, atmospheric temperature and humidity, feeding rate, and the distance between collector plate and spinneret.[18,23] Many groups have investigated how these parameters affect the morphology of the nanofibers produced. Spinning dope viscosity is one of the most important parameters affecting the diameter of the nanofibers produced during electrospinning.[25] Because polymer solution viscosity is dependent on polymer concentration, the higher the polymer concentration the larger the nanofiber diameters become. Demir *et al.* have shown that a power law relationship can be used to model how fiber diameter will increase as polymer concentration is increased, with fiber diameter being proportional to the cube of the polymer concentration.[26] A higher polymer concentration has also been shown to result in less beading on the nanofiber surfaces.[25] The applied electrical

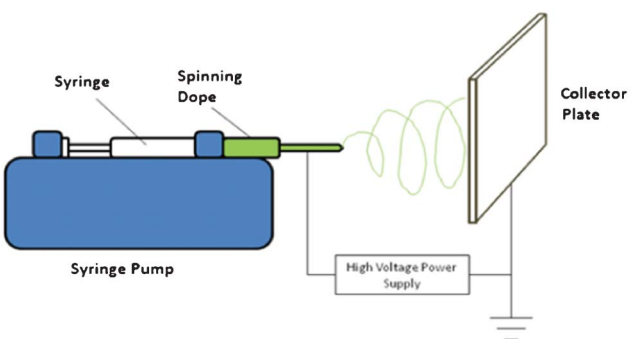

Fig. 1 A basic electrospinning apparatus.

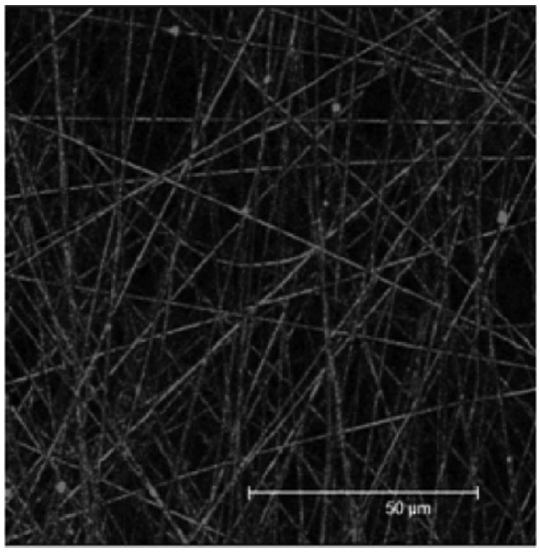

Fig. 2 Confocal microscopy image of poly(vinyl alcohol) electrospun nanofibers.

voltage also has a significant effect on nanofiber diameter. A higher applied voltage causes more fluid to be ejected in a jet, causing larger fiber diameters.[26] The polarity of the electric potential has been shown to have no effect on the spinning process, and fibers can be spun using both negative and positive potentials.[24]

Electrospun nanofibers can easily be functionalized through the incorporation of nanoscale materials within the spinning dope or through surface modifications after spinning (Fig. 3). Conductive nanofibers are frequently fabricated by doping polymer solutions with carbon nanotubes or nanoparticles.[27] Enzymes have also successfully been immobilized within nanofiber networks. Moradzadegan *et al.* created poly(vinyl alcohol) (PVA) nanofibers containing acetylcholinesterase (AChE) by electrospinning a melt of PVA, AChE, and bovine serum albumen (BSA) as an enzyme stabilizer.[28] The AChE modified nanofibers exhibited a 40% activity recovery after electrospinning. Additionally, the enzymes within the nanofibers had a higher stability in acidic solutions when compared to free enzymes. More recently, several groups have looked at the incorporation of molecularly imprinted polymers (MIPs) within nanofiber networks to construct high sensitivity analytical systems (Fig. 3).[22,29] Electrospun polyimide nanofibers imprinted

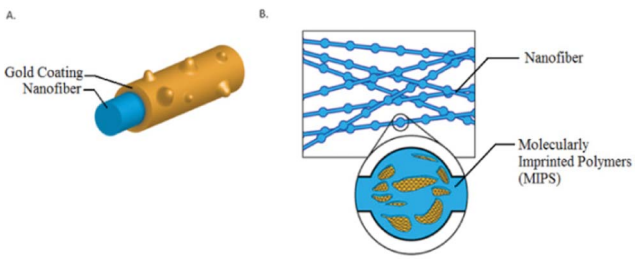

Fig. 3 Functionalization of nanofibers A) Gold-coating of the surface of electrospun nanofibers is performed to improve electron transfer within electrochemical biosensors B) Molecularly imprinted polymers spun within nanofibers to allow for analyte detection.

using a diamine monomer template were able to bind and detect estrone with high sensitivity.[29] The electrospinning of molecularly imprinted nanoparticles within PVA was also able to produce nanofiber mats capable of differentiating between butoxycarbonyl-L-phenylalanine and butoxycarbonyl-D-phenylalanine.[22]

Nanofibers can be fabricated through other methods, such as interfacial polymerization and catalytic synthesis.[18] The fibers produced using these techniques have lengths on the nano to micrometer scale, making them significantly shorter than electrospun nanofibers.[30] Interfacial polymerization is a non-template method of fabrication in which high local concentrations of monomers and dopant anions at a liquid–liquid interface are used to form monomer–anion aggregates.[31] These aggregates serve as nucleation sites for polymerization, ultimately producing nanofiber networks. Interfacial polymerization is often used in the production of polyaniline fibers using organic solvents such as benzene or toluene.[30,31] Nanofiber seeding, in which small amounts of nanofibers are added to a traditional polymerization solution, has been used to increase the efficiency of nanofiber synthesis.[32] In 2004, Zhang *et al.* described a method for synthesizing polypyrrole nanofibers by seeding a polymerization solution with 1–4 mg of 15 mm diameter V_2O_5 nanofibers and noted that the nanofiber production was increased compared to interfacial polymerization methods.[33] Catalytic synthesis is commonly used to fabricate carbon nanofibers.[34,35] Vertically aligned carbon nanofibers were synthesized by Klein *et al.* using a co-sputtered catalysis method.[34] A Cu–Ni composition gradient was used to grow the nanofibers using plasma enhanced chemical vapor etch deposition, yielding fibers with various morphologies based on the percentage of Ni used. Toebes *et al.* used a silica-supported nickel catalyst to produce fishbone carbon nanofibers.[35] The nanofibers produced had uniform morphology and 25 nm diameters. Dilute polymerization is a fiber production method that has also been used to form polyaniline nanofibers.[36] This method produces fibers in an aqueous solution without using templates such as surfactants, large organic dopant acids or nanoscale seeds.[36] The polymerization is carried out in dilute aniline instead of the higher aniline concentration used in other polymerization methods. Chemical vapour deposition has been used to generate carbon nanofibers (Fig. 4). Using this method, fibers can be formed from single-walled and multi-walled carbon nanotubes.[37] The fibers are formed by utilizing a liquid carbon source and iron nanocatalysts to spin fibers on the chemical vapour deposition synthesis zone of a furnace.[37]

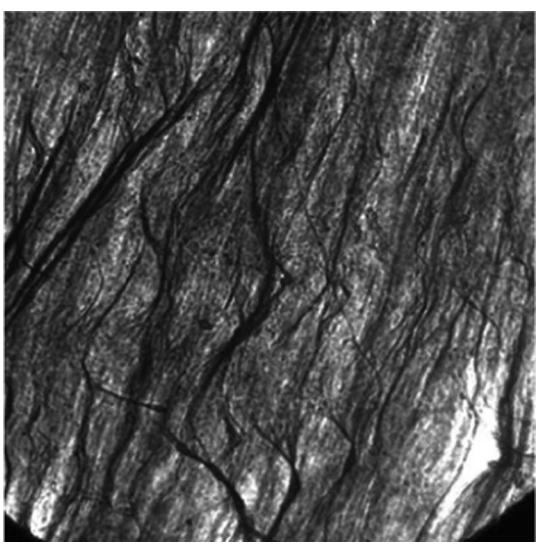

Fig. 4 Carbon nanotube nanofibers formed by chemical vapor deposition. Sample made following the protocol published in Science by Li *et al.*[37] 200×.

Nanofibers are increasingly being incorporated within biosensors to improve the sensitivity and selectivity of analyte detection (Fig. 5). Nanofibers have successfully been used to increase the surface area of electrochemical sensors, provide enhanced sample detection in lateral flow assays, and provide sample concentration within microfluidic sensors. This review looks at the materials most frequently used to form nanofibers for use within sensing systems. We examine the advantages and disadvantages of each material and discuss the effects of nanofiber incorporation on sample preparation and analyte detection.

Applications

Carbon nanofibers

Carbon electrodes have long been used within electrochemical biosensors because they are affordable, biocompatible, and have excellent electron transfer kinetics.[38,39] Carbon nanomaterials, specifically carbon nanotubes, have also been integrated within electrochemical sensors in order to increase the sensitivity of detection.[1,10,11,39–42] In particular, carbon nanotubes offer improved electronic properties and faster electrode kinetics when compared with conventional carbon electrodes.[39] Single walled carbon nanotubes have been used in the design of electrodes for the detection of nucleic acids, cancer biomarkers, neurotransmitters, proteins, and glucose.[10,39] Though carbon nanotubes have successfully been used within biosensors, their commercial viability is currently limited by the fact that their performance is highly dependent on their chirality and diameter, both of which can be difficult to precisely control during synthesis.[1,10] In addition, functionalizing the whole surface of carbon nanotubes can be difficult as ideal carbon nanotubes have chemically inert surfaces with no functional groups.[15,16]

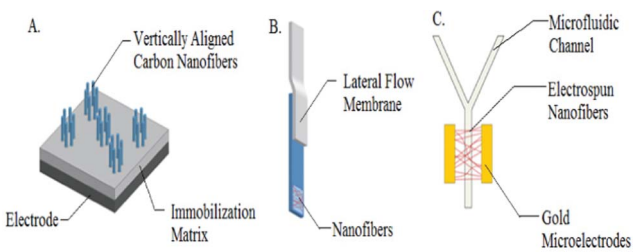

Fig. 5 Examples of nanofibers incorporated within biosensing devices. A) Vertically aligned carbon nanofibers used as nanoelectrodes for electrochemical detection.[40,49] B) Electrospun nanofibers used to increase the surface area of a lateral flow assay.[2] C) Electrospun nanofibers incorporated within a microfluidic device to provide sample preparation and analyte detection.[79,80]

Due to these limitations, many labs are investigating carbon nanofibers as an alternative to carbon nanotubes for highly sensitive analyte detection. Carbon nanofibers have the same high conductivity observed in carbon nanotubes, but can provide an even larger functionalized surface area for the immobilization of biomolecules.[1,43] They can also be easily functionalized along their entire length due to oxygen-containing active sites on their surfaces.[1] In general, carbon nanofibers are cylindrical, consist of graphene layers, and typically have lengths on the order of micrometers.

Vertically aligned carbon nanofibers (VACNFs) are frequently used to create nanoelectrode arrays for analyte detection.[20,44,45] An advantage of these fibers is that they can be individually grown, which allows for a high level of control over the spacing and morphology of VACNF electrodes. In particular, the individual nanofibers can be spaced far enough apart to ensure that the overlapping of radial diffusion layers of adjacent fibers is prevented, but close enough to make densely packed electrode bundles.[40] VACNFs can also be individually functionalized to create heterogeneous electrode bundles.[20,45–48] In 2004, Lee *et al.* presented a method for chemically modifying densely packed VACNF electrode arrays with DNA, proteins, and antibodies.[20] Electrochemical reduction of nitro groups to amino groups on the nanofiber surfaces was used to selectively attach DNA sequences to specific fibers within a 500 nm diameter fiber bundle. Carbon nanotubes were also functionalized with a similar method. McKnight *et al.* demonstrated a method of heterogeneous functionalization of VACNF arrays using photoresist blocking.[45] The VACNFs in this study were functionalized with gold, conductive polymers, DNA and biotin to allow for the capture of enzyme and quantum-dot-conjugated streptavidin. Baker *et al.* developed a method of functionalizing nanofibers through reaction with liquid-phase molecules containing alkene groups.[46,47] Nanofiber arrays modified with primary amines, carboxylic acid groups and alkyl groups were developed. These arrays were successfully used to immobilize cytochrome c for a colorimetric assay.[47]

Though vertically aligned carbon nanofibers can provide a larger functionalized surface area than carbon nanotubes, one common practice is to utilize a matrix to immobilize VACNFs so that only their open ends are exposed on the electrode surface.[40,49] This immobilization serves two purposes. First, it prevents the nanofibers from collapsing upon contact with assay liquids. In addition, exposing only the VACNF ends reportedly increases the sensitivity of the sensors and reduces the occurrence of background "leakage" currents.[49] Consequently, the use of carbon nanotubes and VACNFs may not be significantly different in terms of surface area and functionalization. In addition, the reproducible fabrication of VACNF arrays with uniform fiber heights and densities remains a key challenge to their widespread use, just as with carbon nanotube arrays. In 2009, Arumugam *et al.* attempted to limit variations in nanofiber density by using electron beam patterning on catalyst dots to produce VACNF arrays.[40] The group successfully reduced variations in fiber density, and was able to successfully detect target DNA from *E. coli* O157:H7. However, signal variations attributed to differences in fiber heights were still observed. The group was later able to address the variations in fiber height through the development of an improved electron beam deposition procedure that allowed for the creation of a reproducible electrochemical sensor for the 16 rRNA gene from *E. coli* O157:H7.[49] Despite these advances, several improvements to VACNF electrode design need to be made before they can outperform carbon nanotubes and be used in commercial devices, including improvements to material preparation, probe chemistry, and signal transduction.[49]

More often described is the use of carbon nanofiber mats to modify electrodes for use within electrochemical biosensors[1,41,43] similar to other chemical and polymer modifications frequently used in electrochemistry.[50] These nanofiber films increase the surface functionality of the electrodes and can increase the sensitivity of detection for a variety of different analytes without increasing biosensor size.[43,51,52] Glucose oxidase has been successfully immobilized on carbon nanofiber-modified electrodes to produce high sensitivity glucose biosensors.[1,53] In 2006, Vamvakaki examined different types of carbon nanofibers to determine which were most appropriate for glucose biosensing systems.[1] Their research indicated that graphitized carbon fibers (GFE) had exceptional enzyme loading properties and remarkable stability. The GFE fibers were produced by heat treating basic carbon nanofibers at 3000 °C and consisted of graphene layers arranged in a reversing saw-tooth morphology. The nanofibers were modified with glucose oxidase and maintained their initial activity after 100 h of continuous operation.[1]

When used to modify an electrode, the larger functional surface area of carbon nanofibers can be taken advantage of and improve performance when compared with carbon nanotubes. Wu *et al.* created an electrochemical glucose sensor using carbon nanofibers modified with glucose oxidase and nafion.[53] The immobilization of oxygen-containing groups on the surface of carbon nanofibers was compared to the immobilization of the same groups on carbon nanotubes and the authors report that there were twice as many functional groups on the fibers than on the nanotubes.[53] The sensor had a liner range of 10–350 μM and a limit of detection of 2.5 μM. Its sensitivity was five times higher than many previously reported glucose sensors, including a similar glucose oxidase/titania sol–gel sensor.[54] In addition, the sensor was resistant to interference from a clinically relevant concentration of ascorbic acid (0.08 mM). The concentration of uric acid in human serum samples is generally between 0.18–0.42 mM.[55] Therefore, though the carbon nanofiber sensor was not affected by the interference of 0.08 mM of uric acid, a higher concentration needs to be tested to be truly clinically relevant. However, the sensitivity and stability of the glucose sensor is promising for detection of glucose in clinical samples.

In 2009, Zhang *et al.* reported an amperometric sensor for phenol detection using a polyaniline–ionic liquid carbon nanofiber composite.[41] The composite was formed through electropolymerization of aniline and carbon nanofibers in an ionic liquid. The polyaniline was shown to grow along the carbon nanofibers, resulting in a composite film with fibrillar morphology (95 nm diameter). The composite was used to modify a glassy carbon electrode and was functionalized through the immobilization of tyrosinase on the nanofiber surfaces. The high surface area of the nanofiber film showed a higher tyrosinase immobilization capacity than previously reported devices. The biosensor had a large linear response to catechol detection, ranging from 4.0×10^{-10} to 2.1×10^{-6} M and a limit of detection of 0.1 nM, making it more sensitive than other catechol sensors that do not employ

nanofiber mats.[56] The sensor was unaffected by interference from 3 μM ascorbic acid, 30 μM uric acid, and 30 μM caffeine, which is promising for phenol detection in real samples.

Thionine–carbon nanofibers have been used to create an amperometric ethanol sensor.[43] Electrochemical polymerization was used to form a thionine/carbon nanofiber composite on an electrode surface. The nanofiber film was functionalized with alcohol oxidase and was used to detect ethanol through the reduction of dissolved oxygen. The sensor had a limit of detection of 1.7 μM, which is significantly lower than the 6.26 mM observed in alcohol oxidase immobilization in electrochemically deposited resydrol films.[43,57]

Wu *et al.* have reported an amperometric immunosensor for the detection of carcinoma antigen 125 (CA125) using horseradish peroxidase-labeled carbon nanofibers.[51] The immobilized horseradish peroxidase exhibited good enzymatic activity towards the oxidation of thionine by hydrogen peroxide. The nanofiber-modified biosensor did not require an electron transfer mediator in the solution and therefore required fewer incubation and washing steps than conventional CA125 sensors. The device was used to successfully detect CA125 in standard solutions with a large linear range (2–75 U mL^{-1}) when compared with previously developed sensors, and exhibited a detection limit of 1.8 U mL^{-1}.[58] CA125 detection in serum samples was also carried out and demonstrated comparable results with a commercial electrochemiluminescent assay.

Carbon nanofibers have successfully been used within electrochemical biosensors for a variety of analytes (Table 1). Vertically aligned carbon nanofibers, which can serve as bundles of nanoelectrodes, have been shown to increase the sensitivity of analyte detection when compared with biosensors that do not utilize nanomaterials. However, they can also suffer from the same variable synthesis as carbon nanotubes. In addition, the current method of exposing only the tops of VACNFs fails to take advantage of the high functionalizable surface areas of nanofibers. Carbon nanofibers have also been used to increase the surface area and functionality of electrodes. In these applications, nanofibers have successfully been used to increase the number of functional sites when compared to nanotubes or non-nanoscale materials. Biosensors utilizing carbon nanofibers improved the sensitivity of glucose, catechol, and ethanol detection. In addition, the nanofibers dramatically increased the linear range for CA125 detection.

Polyaniline nanofibers

Conductive polymers, like polyaniline (PANI), are frequently used as immobilization matrices for enzymes within electrochemical biosensors.[59] The PANI matrix provides a porous medium for immobilization and facilitates electron transfer between enzymes and electrodes. PANI nanostructures have also been successfully utilized in electrochemical biosensors.[9] Berti *et al.* utilized PANI nanotubes to modify an electrode surface through electrochemical polymerization with alumina nanoporous membranes as a mold. These nanostructures were grafted with molecularly imprinted polymer receptors to create a catechol biosensor.[9] Nano-structured polyaniline films have also successfully been used to immobilize glucose oxidase to facilitate electrochemical detection.[60]

Polyaniline nanofibers are also frequently used to increase the sensitivity and conductivity of electrochemical biosensors.[61,62] Compared to conventional PANI materials, PANI nanofibers have the advantage of being inexpensive, easy to produce, and have a much larger surface area.[61] However, PANI's redox activity is generally restricted to acidic environments, limiting its use in biological systems, which frequently are neutral pH environments.[62] Therefore, self-doped polyaniline (SPAN) is also utilized within nanofibers. SPAN is produced through copolymerization of aniline and *m*-aminobenzenesulfonic acid in an aqueous solution and features better activity and stability at neutral pH.[13,63,64]

Polyaniline nanofibers are often used to modify glassy carbon electrodes for enzyme immobilization because of their conductivity and electroactivity. In particular, hydrogen peroxide sensors utilizing PANI nanofibers have recently gained significant attention. In 2009, Du *et al.* described a simple electrode modification method in which a mixture of PANI/chitosan nanofibers and horseradish peroxidase were dropped onto a glassy carbon electrode to produce a hydrogen peroxide biosensor.[61] The nanofibers were fabricated using interfacial polymerization with 4-toluenesulfonic acid as a dopant. The PANI nanofibers were dispersed in a chitosan solution to improve nanofiber stability. The immobilized horseradish peroxidase was shown to keep its native activity and successfully reduced H_2O_2. The device had a wide linear range of 1×10^{-5} to 1.5×10^{-3} M and a low limit of detection of 5×10^{-7} M. Recently, Chen *et al.* incorporated gold nanoparticles within SPAN nanofibers and immobilized horseradish peroxidase on the nanofiber surfaces to create a sensitive H_2O_2 sensor.[13] The gold nanoparticles served to increase the conductivity and biocompatibility of the SPAN nanofibers. The increased number of enzyme immobilization sites resulted in increased electrocatalytic activity in the reduction of H_2O_2 in the presence of hydroquinone. The sensor was used for successful detection of H_2O_2 in real contact lens solution samples and results were comparable to those obtained by conventional potassium permanganate titration. These two PANI nanofiber biosensors

Table 1 Comparison of linear range and limit of detection for nanofiber-based and conventional biosensors

Sensor materials	Analyte	Linear range	Limit of detection
Carbon nanofibers[53]	Glucose	10–350 μM	2.5 μM
GO_x/titania sol–gel[54]	Glucose	70–15 000 μM	70 μM
Polyaniline/carbon nanofiber composit[41]	Catechol	4.0×10^{-4}–2.1 μM	0.0001 μM
Polyaniline/polyphenol oxidase film[56]	Catechol	2.5–140 μM	0.05 μM
Thionine/carbon nanofiber[43]	Ethanol	2.0–252 μM	1.7 μM
Resydrol film[57]	Ethanol	Not reported	6,260 μM
Peroxidase-labeled carbon nanofibers[51]	CA125	2–75 Unit mL^{-1}	1.8 Unit mL^{-1}
Peroxidase film[58]	CA125	2–14 Unit mL^{-1}	1.29 Unit mL^{-1}

allowed for sensitive detection of hydrogen peroxide, but their performance was not better than a similar PANI/nanotube sensor (Table 2). However, when compared to a sensor composed of a thin polyaniline film on a platinum disc electrode, the PANI nanofiber sensors had a dramatically lower limit of detection.[65] This demonstrates the benefits of the larger surface area provided by nanomaterials such as nanofibers and nanotubes.

Polyaniline nanofibers have also been used to increase the sensitivity of DNA detection.[3,62,63] In 2011, Wang *et al.* utilized three-step electrodeposition to create self-doped polyaniline nanofibers patterned with Au microspheres.[62] The nanofibers were used to modify a glassy carbon electrode in order to detect a gene fragment from the cauliflower mosaic virus 35S gene. The limit of detection observed (1.9×10^{-14} M) was lower than previously reported non-nanofiber based DNA sensors.[67] ZrO_2 microparticles have also been used to create SPAN nanofiber membranes for DNA sensing on glassy carbon electrodes.[63] An ssDNA sequence was immobilized to the ZrO_2/SPAN/electrode surface to allow for the detection of target DNA. The ZrO_2 microparticles have a high affinity for the oxygen containing groups on the nanofibers and therefore could be electrochemically deposited on nanofiber surfaces using cyclic voltammetry. The sensor also demonstrated a very low limit of detection (3.4×10^{-13} M), good specificity for target DNA and did not detect one base pair mismatch DNA sequences or non-complementary DNA. Spain *et al.* also demonstrated DNA detection using PANI nanofibers modified with gold nanoparticles on a gold electrode surface.[3] This device utilized the enzyme immobilization properties of PANI to immobilize horseradish peroxidase on the surface of the nanofibers. The nanoparticles were used to immobilize ssDNA complementary to a target strand of DNA from *Staphylococus aureus*. Hybridized target DNA was detected using the reduction of hydroquinone to mediate electron transfer to bound horseradish peroxidase. The device was able to successfully differentiate between *S. aureus* and *S. epidermidis*, indicating a low false positive rate that makes it a promising option for detection in real samples. In addition, the sensitivity of detection was 40-fold greater than detection using a bare electrode surface.[3]

The successful incorporation of PANI and SPAN nanofibers within DNA sensors shows great promise for the development of highly sensitive genetic sensing. The increased surface area provided by the nanofibers resulted in a dramatic increase in the linear range when compared to non-nanofiber based sensors (Table 3). In addition, the limits of detection for PANI and SPAN nanofiber sensors were significantly lower than their conventional counterparts.

Chitin/chitosan nanofibers

Chitin and its derivative chitosan are biodegradable and biocompatible polymers derived from the exoskeletons of arthropods and the cell walls of yeast and fungi.[14,68] Chitosan is an excellent substrate for enzyme immobilization and can easily be electrospun into high surface area nanofiber mats. In addition, chitosan nanofibers exhibit high mechanical strength, hydrophilicity, and exceptionally small pores size when spun into mats.[14,68] Chitin, on the other hand, is a difficult material to work with and does not dissolve in most common solvents. However, both chitin and chitosan nanofibers have been successfully used in many applications, such as drug release, tissue engineering, and wound healing.[14] Chitosan has traditionally been used to immobilize enzymes within biosensors due to the amino group and two hydroxyl groups in each molecular unit that can easily be crosslinked within different substances.[69,70] Chitosan/$NiFe_2O_4$ nanoparticles have also been used to immobilize glucose oxidase for electrochemical detection in a glassy carbon electrode biosensor.[71] Finally, three-dimensional chitosan membranes have also been utilized to increase electrode surface areas for electrochemical detection.[72]

Recently, chitin and chitosan nanofibers have been incorporated within biosensing devices to take advantage of their excellent enzyme immobilization properties. An amperometric cholesterol biosensor consisting of cholesterol oxidase (ChO_x) immobilized on a chitosan nanofiber/gold nanoparticle network has been developed by Gomathi *et al.* The nanofibers had diameters ranging from 50–100 nm and were prepared by oil/water emulsion.[68] The gold nanoparticles were electrochemically deposited on the nanofibers from a $HAuCl_4$ solution. The device was able to reproducibly measure cholesterol within real human serum samples and did not respond to clinically relevant concentrations of ascorbic acid and uric acid in PBS.[68] The limit of detection (0.5 μM) was also substantially lower than the limit of detection observed in non-nanofiber based sensors (Table 4).[73] Chitosan/poly(vinyl alcohol) electrospun nanofibers have also been used for enzyme immobilization within biosensors.[74] The nanofibers had diameters ranging from 80–150 nm and were utilized for enzyme immobilization due to their biocompatibility and porosity. The enzymes were used to immobilize lipase from *Candida rugosa* using glutaraldehyde as a coupling reagent. The immobilized enzyme retained 49.8% of its activity and had improved thermal and pH stability when compared to free enzyme.

Poly(vinyl alcohol) nanofibers

Poly(vinyl alcohol) (PVA) is a water-soluble, biocompatible polymer that has excellent fiber formation properties.[75] Unlike many other polymers, PVA has the advantage of being able to be electrospun using water as a solvent and can be easily stabilized through cross-linking of the free-hydroxyl groups on the fiber surfaces.[22] The hydroxyl groups can also be used to easily functionalize the nanofibers. Generally, PVA membranes have

Table 2 Comparison of PANI sensors for hydrogen peroxide

Sensor materials	Analyte	Linear range	Limit of detection
Polyaniline nanofiber/chitosan film[61]	Hydrogen peroxide	10–1500 μM	0.5 μM
Polyaniline nanotube/chitosan nanocomposite[66]	Hydrogen peroxide	1.0–2200 μM	0.5 μM
Gold nanoparticle/SPAN nanofiber[13]	Hydrogen peroxide	10–2000 μM	1.6 μM
Polyaniline film[65]	Hydrogen peroxide	250–5000 μM	250 μM

Table 3 Comparison of polyaniline nanofiber and polyaniline matrix DNA biosensors

Sensor materials	Analyte	Linear range	Limit of detection
SPAN nanofiber/Au Microspheres[62]	Cauliflower mosaic virus	1.0×10^{-7}–1.0 μM	1.9×10^{-8} μM
Nanogold modified poly-2,6-pyridinedicarboxylic acid film[67]	PAT gene	1.0×10^{-4}–10 μM	2.4×10^{-5} μM
ZrO_2/SPAN nanofiber/carbon electrode[63]	ssDNA	1.0×10^{-6}–1.0 μM	3.4×10^{-7} μM
Au nanoparticle/polyaniline nanofiber[3]	*S. aureus*	150×10^{-6}–1.0 μM	pM range

Table 4 A comparison of chitosan nanofiber and chitosan film cholesterol biosensors

Sensor materials	Analyte	Linear range	Limit of detection
Chitosan nanofiber/gold nanoparticles[68]	Cholesterol	1–45 μM	0.5 μM
Metal oxide/chitosan composite film[73]	Cholesterol	10–400 mg dL^{-1}	5 mg dL^{-1}

been used to immobilize enzymes within electrochemical biosensors.[76] In addition, PVA has been used to modify carbon nanotubes for application within electrochemical biosensors.[77] The PVA serves as a binder that permits immobilization of biomolecules on the nanotube surfaces.[77]

PVA nanofibers have been used as supports for molecularly imprinted polymer (MIP) nanoparticles for the detection of dansyl-L-phenylalanine.[22] Molecularly imprinted polymers are traditionally immobilized onto solid surfaces, which results in low surface areas and binding capacities. Therefore, nanofiber mats have been investigated to create a higher surface area for analyte detection. The MIPs had a diameter of 400 nm and were contained within nanofibers with diameters between 80–350 nm to ensure that the binding sites of the nanoparticles were not completely covered by the fibers. Fluorescent microscopy confirmed the binding of dansyl-L-phenylalanine to the MIPs with no nonspecific binding of the analyte to the fibers.

PVA nanofibers have also been used for enzyme immobilization in amperometric biosensors.[78] The nanofibers were used to immobilize glucose oxidase to allow for the sensitive detection of glucose. Chronoamperometric measurements showed that the nanofiber modified electrodes demonstrated a rapid response (1 s) and had a good detection response (μA level) to both normal and diabetic levels of glucose. The device had a linear range from 1–10 mM and a detection limit of 0.05 mM. This limit of detection is lower than the limits of detection observed in some non-nanofiber sensors,[54] but is higher than carbon-nanofiber sensors that have been developed.[53]

Recently, Cho *et al.* demonstrated the successful incorporation of electrospun PVA nanofibers within microfluidic systems to allow for sample preparation as well as analyte detection within microfluidic devices.[79] Positively charged PVA fibers were fabricated through the incorporation of hexadimethrine bromide within the polymer spinning dope, while negatively charged fibers were produced using poly(methyl vinyl ether-alt-maleic anhydride). These fibers were spun on gold microelectrodes patterned on poly(methyl methacrylate) (PMMA). The PMMA pieces patterned with microelectrodes and nanofibers were bonded to PMMA pieces embossed with microfluidic channels using thermal bonding on a hot press. The fibers were shown to maintain their morphology in fluid flow up to 20 μL min^{-1}. Positively charged nanofibers were shown to successfully filter negatively charged nanoparticles out of a buffer solution, allowing for sample concentration and purification within a microchannel.[80]

Other materials

Polylactic acid (PLA) is commonly used in the electrospinning of nanofibers for a variety of applications from tissue engineering to drug delivery.[81] In 2006, Li *et al.* utilized electrospinning to produce biotinylated nanofiber membranes that provided an extremely large number of binding sites for streptavidin.[81] Nanofibers were produced by dispersing biotin in a PLA/chloroform/acetone solution before electrospinning. Electron probe microanalysis confirmed the presence of biotin on the surface of the electrospun fibers. Additional analysis confirmed that biotin was fixed to the fiber surfaces and was not washed off during fluid flow. A basic biosensor was constructed using the nanofiber membrane to immobilize biotinylated nucleic acid probes for detecting synthetic *E. coli* DNA.[81,82]

Polypyrrole (PPy), like polyaniline, is a naturally conducting polymer, and has been used within electrochemical DNA sensors.[83] Unlike polyaniline, PPy can be synthesized using neutral pH aqueous solutions. The polymer nanofibers were grown on platinum electrodes and were synthesized through electropolymerization of pyrrole using pulse voltammetry. The device was used to detect low concentrations of spermidine with a limit of detection of 0.02 μM. Polypyrrole nanofibers have also been used to modify electrodes for the detection of salicylic acid and aspirin.[84] Double stranded calf thymus DNA was physisorbed onto PPy nanofibers on a platinum electrode. The device showed a limit of detection of 8.26×10^{-1} and 5.24×10^{-6} μM for salicylic acid and aspirin respectively.

Conclusions

The high surface area provided by nanofiber arrays and mats has been shown to dramatically increase the sensitivity of many biosensors. Carbon and polyaniline have gained the most attention due to their conductivity, biocompatibility, and long history of use within biosensors. However, many other materials, such as polypyrrole and chitosan, have been successfully used to form nanofibers for improved detection for a wide variety of analytes.

Vertically aligned carbon nanofibers have been shown to increase the sensitivity of electrochemical biosensors. Moreover, several labs have demonstrated the ability to functionalize individual nanofibers within a VACNF bundle, allowing for the creation of heterogeneous nanofiber electrodes. Currently, VACNFs are often immobilized within a matrix that serves as a

support and reduces the background signals observed within the sensors. However, as only the tops of the nanofibers are available for interaction with the analyte, there is no significant increase in functional surface area when compared to carbon nanotubes or other nanomaterials. In addition, the reproducible synthesis of VACNF bundles can be difficult, resulting in variations in signal from sensor to sensor. Consequently, improvements to material synthesis and fiber morphology should be completed to standardize sensor behavior.

Carbon, polyaniline, chitosan, poly(vinyl alcohol), polypyrrole and polylactic acid nanofibers have also been used to increase the surface area of electrodes within electrochemical sensors. The fibers have been shown to significantly increase the available functionalized surface area on the electrode and can result in larger linear ranges and lower limits of detection when compared to other sensors. Novel nanofiber-based biosensors are continually being reported in the literature, though generally the ability of these sensors to detect analytes within real clinical, environmental, and food safety samples is not significantly discussed. Further studies on sample preparation and analyte detection within nanofiber-based biosensors need to be conducted before they can be used in commercial sensors.

Acknowledgements

The authors wish to acknowledge the support provided by NSF CBET-0852900. In addition, the authors are thankful for the financial aid provided by the Cornell Center for Materials Research "Materials for a Sustainable Future" IGERT program provided though the National Science Foundation grant number DGE-0903653. The authors would also like to thank Andrei Georgescu for his invaluable help creating the figures for this article. Finally, the authors would like to acknowledge Dr Daehwan Cho for providing the carbon nanotube nanofibers imaged in this work.

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