Biologically Inspired Nanofibers for Use in Translational Bioanalytical Systems

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Abstract

Electrospun nanofiber mats are characterized by large surface-area-to-volume ratios, high porosities, and a diverse range of chemical functionalities. Although electrospun nanofibers have been used successfully to increase the immobilization efficiency of biorecognition elements and improve the sensitivity of biosensors, the full potential of nanofiber-based biosensing has not yet been realized. Therefore, this review presents novel electrospun nanofiber chemistries developed in fields such as tissue engineering and drug delivery that have direct application within the field of biosensing. Specifically, this review focuses on fibers that directly encapsulate biological additives that serve as immobilization matrices for biological species and that are used to create biomimetic scaffolds. Biosensors that incorporate these nanofibers are presented, along with potential future biosensing applications such as the development of cell culture and in vivo sensors.

INTRODUCTION

Bioanalytical systems rely on the specific and sensitive binding of a biorecognition molecule with its analyte, such as glucose oxidase with glucose, an antibody with its antigen, and a DNA oligonucleotide with its complementary sequence. Together with the choice of biorecognition molecule and signal transduction principle, the sensor surface is the characteristic that has the most dramatic influence on sensor performance. Providing a hindrance-free and recognition site–concentrated surface is critical and is generally accomplished through the immobilization of the biorecognition molecule on a planar surface. One-dimensional, nanoscale materials can be used to improve the performance of these devices by dramatically increasing the surface area available for detection (1–4). Because the sensitivity of a sensor is related to the number of detection sites available, it is beneficial to provide an increase in functionalized surface area without increasing the sample volume needed (5). Nanoscale materials have inherently large surface-area-to-volume ratios and provide a marked improvement over conventional materials such as polymer films and fibrous membranes, which have limited surface areas and low loading capacities (6). Additionally, nanoscale materials can be made with a wide range of surface chemistries and mechanical properties, making them ideally suited for use in biosensing devices (2, 3, 7).

Nanofibers (4, 8, 9), nanowires (3, 10, 11), nanotubes (1, 3), and nanoparticles (1, 11) have been successfully incorporated into sensing devices, improving their sensitivity and efficiency. Of these materials, electrospun nanofibers stand out for their porous, nonwoven structure. The high porosity and surface area of electrospun nanofiber mats provide excellent loading capacities for immobilization of biological molecules and allow for an improved mass-transfer rate for a substrate to functionalized sites within the mat (12). Nanofiber mats are durable, easy to handle, reusable, and appropriate for use within microfluidic devices (6, 13). In addition, electrospun nanofibers are easy to fabricate and functionalize (7, 14). Of the different fiber-formation processes that can be used to produce nanofibers, electrospinning is arguably the best-suited method for the mass production of continuous fibers with uniform diameters (7). In addition to straightforward fabrication, electrospinning allows for the creation of fibers from materials that could not be used easily in other fiber-formation processes (15). For example, proteins cannot be incorporated easily into fibrous forms because of their complex three-dimensional structure and strong inter/intramolecular forces (15). However, several (9, 15, 16) have reported on electrospun nanofibers made from proteins, such as bovine serum albumin (BSA) and casein, which are electrospun by coupling the proteins with a second, more easily spun polymer.

Scope and Structure of Review

Although some significant papers have demonstrated highly successful integration of biologically functionalized nanofibers into sensing platforms (4), the field is in its infancy and we must learn from other disciplines, such as tissue engineering, to expand the types of materials available for use in biosensors (17, 18). Recently, there have been many exciting advances in the types of nanofibers that can be produced by electrospinning, and translational studies are needed to bring these findings to full bear in bioanalytical systems. Therefore, this review begins with a discussion of novel nanofiber chemistries, focusing on fibers created by incorporating biological additives into spinning dopes (8, 9, 12, 15), fibers used as immobilization matrices for biological species (19–21), and fiber scaffolds used to duplicate the structure of biological materials (17, 22, 23). Then, we present potential applications of these novel nanofiber types in biosensing. Specifically, we discuss the creation of more sensitive and efficient biosensors, biomimetic biosensors for use in cell culture analysis, and biocompatible and reagentless in vivo biosensors.

ELECTROSPINNING

Nanofibers can be fabricated by a variety of methods, such as interfacial polymerization, catalytic synthesis, and self-assembly (4, 24). However, these methods each have their limitations. On the one hand, they can have slow production rates, expensive fabrication, restricted ranges of usable materials, and few possible nanofiber morphologies (24). On the other hand, electrospinning is a relatively simple, high-throughput process in which electrical forces are used to form nanofibers with uniform diameters and long lengths from a viscous polymer solution (4, 14, 24). Unlike many other fiber-formation methods, electrospinning can be used to create fibers made from an extremely wide range of materials, including biological polymers (25, 26), globular proteins (9, 16), and conductive materials (27). Furthermore, several types of fiber morphologies can be made by electrospinning, including beaded, ribbon-shaped, and core-shell fibers (24).

A typical electrospinning apparatus is composed of a spinneret (typically a syringe), a spinning dope, a high voltage source, and a grounded collector plate (14) (**Figure 1**). Often, a syringe pump is used to slowly push the spinning solution out of the spinneret. The high voltage source is connected to the tip of the spinneret and confers a constant charge to the spinning solution, causing the solution to form what is referred to as the Taylor cone at the tip of the spinneret (4, 14). The spinning dope will accelerate out of the tip of the Taylor cone once the electrostatic attraction between the grounded collector plate and the charged solution overcomes the surface tension at end of the syringe tip (28). As the polymer jet travels from the spinneret to the collector plate, it undergoes whipping, and the solvent in the jet evaporates, leaving behind a solid polymer fiber that collects as a nonwoven mat on the grounded collector plate. These nanofiber mats are characterized by extremely large surface-area-to-volume ratios, high porosity, and small pore sizes (29).

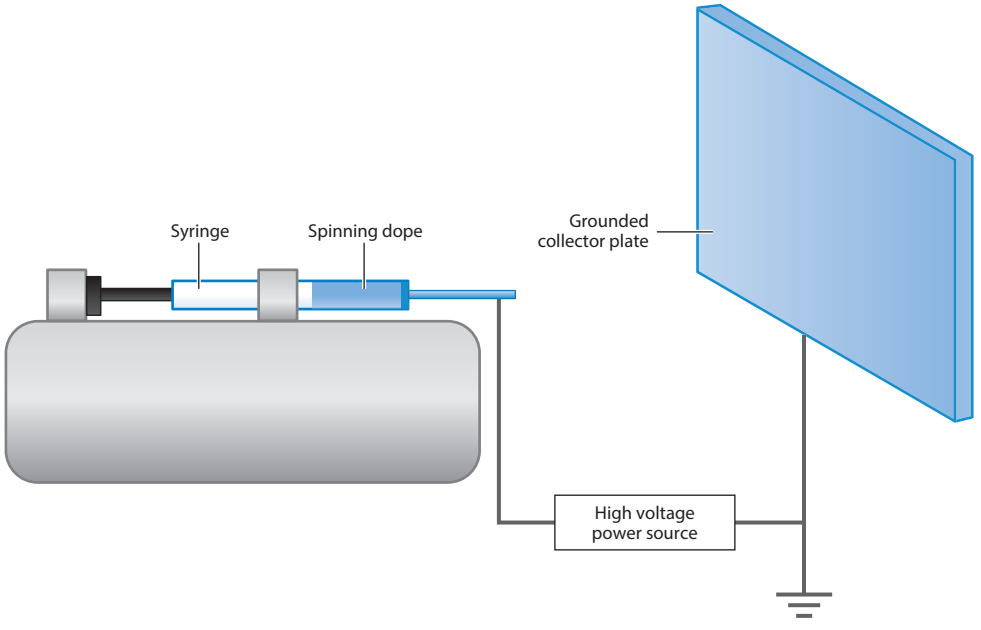


Figure 1

A typical spinning apparatus consisting of a syringe pump, spinneret, high voltage power source, and grounded collector plate.

Electrospun nanofibers can easily be functionalized, either through postspinning chemical modifications or by directly incorporating nanoscale materials into the spinning dope. Nanofiber mats can be modified using standard chemical coupling strategies to immobilize biological species such as enzymes (19) and antibodies (22). Often, the immobilization efficiency on nanofiber mats is dramatically improved when compared to conventional materials (12). The process of incorporating biological species into a spinning dope is a more elegant functionalization of the nanoscale material, as no postprocessing is required. However, it is also a more complex procedure given that spinning conditions have to be tailored toward conditions acceptable for the biomolecule to be immobilized. Examples of successful biomolecule incorporation include nanofibers containing biotin (6, 30), antibodies (8), and enzymes (15). These biological materials retain their native function and can have increased stability due to their immobilization within the mats (12).

CREATION OF BIOFUNCTIONAL NANOFIBERS

Many biological species have been incorporated successfully within electrospun nanofiber mats. These materials can be directly spun into fibers, coupled with secondary polymers that enhance their spinnability, or they can be immobilized on nanofibers postspinning. Regardless of the fabrication method used, the high surface area of electrospun nanofiber mats results in an increase in the number of immobilization sites available when compared to conventional materials.

Globular Proteins

Globular proteins are difficult to process into fibrous forms because the proteins do not have the same viscoelastic properties as classic polymers due to the proteins' strong inter- and intramolecular forces (15, 31, 32). To overcome these limitations, globular proteins can be co-electrospun with another polymer, which serves to reduce the inter- and intramolecular bonding of the proteins and results in successful production of nanofibers (9, 15, 16, 33). However, it is also possible to spin the proteins without the use of a second polymer (31, 32). Currently, globular protein-based nanofibers are primarily utilized within tissue engineering scaffolds; however, they can also be easily functionalized to produce biocompatible, stable fibers for use in cell culture analysis or in vivo sensing (9) (**Table 1**). In addition, functionalized casein- or BSA-based nanofibers could be utilized to prevent nonspecific binding on fiber surfaces, eliminating or reducing the need for additional blocking steps within biosensing systems.

BSA is conventionally used as a blocking agent, enzyme stabilizer, and tissue culture nutrient (9, 16). Generally, it has proven difficult to spin without the use of a second polymer such as poly(ethylene oxide) (PEO) (9) or poly(ethylene glycol) (PEG) (16). Kowalczyk et al. (9) produced a simple pH sensor from electrospun BSA fibers stained with fluorescein isothiocyanate (FITC) for use in cell culture analysis. A water-resistant PEO core was used to increase the stability and spinnability of the fibers. Directly after spinning, the PEO/BSA fibers were water soluble, but after aging for two weeks at ambient conditions they were rendered insoluble and retained their structure in solution (9). FITC was coupled to the fiber surfaces by immersing the mats in a 0.02wt% FITC solution for 24 h at room temperature. The biocompatibility of the resulting fiber and simplicity of its pH response make it a desirable candidate for use as an intracellular pH monitor. Zhang et al. (16) report a slightly more complex approach in which coaxial electrospinning was used for the production of BSA nanofibers. Here, water-soluble PEG, FITC-conjugated BSA (fitcBSA), and biodegradable poly(ε-caprolactone) (PCL) were coaxially spun to create a biologically mimetic tissue engineering scaffold capable of sustained release of fitcBSA. The fiber composition was controlled to prevent an initial burst release of fitcBSA. Similarly, a polycaprolactone and BSA

Table 1 Electrospinning of globular proteins

Biological additive	Spinning method	Copolymer(s) spun	Demonstrated application	Citation	Additional potential bioanalytical use
BSA	Secondary polymer support	PEG FitcBSA PCL	Tissue engineering scaffold	16	Integrated sensing moieties Release of cofactors for catalytic biosensors
BSA	Secondary polymer support	Polycaprolactone	Tissue engineering scaffold	33	Release of cofactors for catalytic biosensors
BSA	Spinning	NA	NA	31	Biostabilization for biorecognition elements
Casein	Secondary polymer support	PEO PVA	Enzyme immobilization	15	Catalytic biosensors
Hemoglobin and myoglobin	Spinning	NA	Oxygen delivery wound-healing scaffold	32	High-sensitivity electrochemical biosensors
Hemoglobin	Spinning	NA	H_2O_2 and nitrite biosensor	34	High-sensitivity electrochemical biosensors

Abbreviations: BSA; bovine serum albumin; PCL, poly(ε-caprolactone); PEG, poly(ethylene glycol); PEO, poly(ethylene oxide); PVA, poly(vinyl alcohol).

nanofiber has been developed for use in peripheral nerve tissue engineering scaffolds (33). Nerve growth factor was successfully encapsulated within the fibers, resulting in a nanofiber mat that mimicked the structure of the extracellular matrix while also providing an increased surface area for release of nerve growth factor. The nanofibers had a reduced initial burst release of nerve growth factor and an improved loading efficiency when compared to other hydrophobic polymers. The nerve growth factor was consistently released for 28 days and was capable of inducing neutrite outgrowth from PC12 cells grown on the scaffold (33).

Dror et al. (31) were able to spin BSA fibers without the use of a second polymer support by manipulating the BSA's existing disulfide bonds to allow for the formation of a structure rich in inter- and intramolecular disulfide covalent bonds. A high concentration of trifluoroethanol (TFE) was used to disrupt the tertiary structure of the BSA to get an open helical structure with many hydrophobic protein segments exposed to the solvent. β-Mercaptoethanol was used to open the intramolecular disulfide bridges of the BSA, producing a marked improvement in the rheological properties and spinnability of the solution. The resulting BSA fibers had uniform morphologies, high tensile strength, and high degrees of crystallinity, making them well-suited for applications in tissue engineering or sensing (31).

Hemoglobin and myoglobin are redox proteins used within electrochemical biosensors (34, 35). Generally, the proteins are immobilized within a matrix, such as a polyacrylamide film (36), or immobilized onto the surface of nanoparticles and nanotubes before they can be used within a biosensor (35, 37). Recently, however, the feasibility of spinning both myoglobin and hemoglobin nanofibers has been established, allowing for the creation of protein-based biosensors that do not require complicated immobilization procedures (32, 34). Myoglobin and hemoglobin have both been successfully spun into ribbon-like nanofibers without the use of a second polymer (32). The proteins were solubilized in 2,2,2-trifluoroethanol prior to spinning. Nanofibers were spun with hemoglobin concentrations between 100 and 250 mg/ml and a myoglobin concentration of 250 mg/ml. The fibers were cross-linked to improve stability through vapor fixation with a 50%w/w glutaraldehyde solution. Although the mats were stable when handled gently, they

lacked the mechanical strength to withstand harsh conditions. However, both fiber mats were deemed sturdy enough for use as a potential biocompatible wound dressing (32). Hemoglobin has also been electrospun to produce a biosensor for the detection of hydrogen peroxide and nitrite (34). The hemoglobin was spun onto a glassy carbon electrode surface to create an electrochemical sensor. The hemoglobin retained its activity after spinning and required no immobilization matrix to adhere to the glassy carbon electrode, making it an attractive alternative to conventional immobilization. The fibers were spun from 175 mg/ml hemoglobin in 2,2,2-TFE solution and were cross-linked in glutaraldehyde vapor after spinning to render them insoluble. An amperometric sensor created from the fibers showed limits of detection of 0.61 μM for H_2O_2 and 0.47 μM for nitrite (34).

Casein is typically used as a blocking agent to prevent nonspecific binding within biosensors. As with BSA, casein can be difficult to spin without the support of a second polymer and has been successfully spun through incorporation in a PEO and poly(vinyl alcohol) (PVA) spinning dope (15). Furthermore, lipase enzyme was added to the spinning solution to create a functionalized nanofiber. Lipase immobilized within the casein fibers demonstrated a higher catalytic activity toward the hydrolysis of olive oil than lipase immobilized on planar materials (15). The resulting fiber could be used to lower the nonspecific binding within a sensing device and to simultaneously permit enhanced enzyme-based detection of analytes.

Enzymes

Enzymes are used as catalytic biorecognition molecules in bioanalytical sensors. Maintaining an enzyme's tertiary structure during immobilization/spinning is necessary to maintain its catalytic activity. Several groups have reported the successful integration of enzymes into electrospun nanofiber mats, either by adding the enzymes to a polymer spinning dope or through postspinning immobilization on the mats (**Table 2**). The enzyme-based nanofibers have several advantages over conventional enzyme immobilization strategies, including increased immobilization efficiency due to the large surface area of the nanofibers, improved mass transfer for the substrates to

Table 2 Immobilization of enzymes within nanofiber mats

Biological additive	Immobilization method	Additional polymer(s) spun	Results	Citation	Potential bioanalytical use
Lipase	Cospinning with casein and secondary polymer	PEO PVA	Increased enzyme activity	15	Prevention of nonspecific binding
Lipase	Adsorption on coaxial-spun fibers	PMPPh PAN	Increased enzyme stability	39	Long-term storage Continuous/long-term measurement sensors
β-galactosidase	Covalently attached via carbonyl groups of spacer arms	Poly(AN-*co*-MMA)	Better activity Better temperature stability	19	Sensors for use at extreme temperatures
Glucose oxidase	Postspinning	Chitosan PVA	Sensor with improved stability in neutral and alkaline conditions	21	Sensors modified for use under extreme pH conditions

Abbreviations: PAN, polyacrylonitrile; PEO, poly(ethylene oxide); PMPPh, poly[bis(*p*-methylphenoxy)]phosphazene; poly(AN-*co*-MMA), poly(acrylonitrile-*co*-methyl methacrylate); PVA, poly(vinyl alcohol).

the enzyme reactive sites, improved long-term enzyme stability, higher enzyme activities than enzymes immobilized in films, and simpler immobilization procedures (12).

Enzymes can be spun successfully into nanofiber mats by incorporating them into polymer spinning dopes (12, 15, 38). As previously discussed, lipase has been cospun into casein nanofibers by using PEO and PVA to improve the spinnability of the proteins (15). The immobilized lipase had greater ability to hydrolyze olive oil than did lipase immobilized in films. Herricks et al. (12) successfully spun α-chymotrypsin by incorporating it into poly(styrene-*co*-maleic anhydride) (PSA) dissolved in dioctyl sulfosuccinate-toluene solution. The nanofibers were stabilized using glutaraldehyde, resulting in fibers that were stable in solution. α-Chymotrypsin immobilization within the fibers resulted in greater enzyme stability compared to immobilization in conventional materials such as bulk films. Additionally, there was an increased mass-transfer rate for the substrates to the enzyme reactive sizes due to the morphology of the nanofiber mats. The stable α-chymotrypsin fibers were almost five times as active as glutaraldehyde treated α-chymotrypsin films (12). A water-soluble polyvinylpyrrolidone (PVP) nanofiber has been created to facilitate storage of horseradish peroxidase (HRP) within microfluidic biosensors (38). The HRP was spun directly into the fibers, resulting in better enzyme activity after long-term storage in ambient conditions and easier integration of HRP into microfluidic sensors. The immobilized HRP retained 80% of its native activity after spinning and retained 40% activity after storage for 280 days at ambient conditions. The spinning of HRP into the fibers eliminated the need for lyophilization of the enzymes and is an attractive option for the immobilization of other biological recognition elements in point-of-care sensors (8, 38).

Enzymes are also immobilized on nanofibers through postspinning chemical coupling (19, 39). The shared goal among the reports is to increase the surface area of available enzyme and allow for better mass transfer of substrate to enzyme active sites. Wang et al. (39) report coaxial-electrospun fibers with a poly[bis(*p*-methylphenoxy)]phosphazene (PMPPh) sheath and polyacrylonitrile (PAN) core. The PAN was used to facilitate better fiber formation of the PMPPh. The resulting fibers were able to immobilize lipase via adsorption. The core/sheath fiber had better adsorption capacity (20.4 ± 2.7 mg/g) and increased enzyme activity (63.7%) than lipase immobilized on a PAN membrane. The organic side groups of the polyphosphazene allowed for easy nucleophilic substitute and creation of a wide range of side groups that can be used to immobilize different biological recognition elements. In contrast to this adsorptive approach, β-galactosidase has also been immobilized onto nanofiber mats via covalent bonding in an effort to improve enzyme stability (19). Poly(acrylonitrile-*co*-methyl methacrylate) [poly(AN-*co*-MMA)] fibers were spun and modified with a polyethyleneimine (PEI) spacer arm that was used to covalently attach the β-galactosidase to the carbonyl groups on the fibers. Glutaraldehyde was used as a coupling agent. After immobilization, the enzyme stability was improved (V_{max} = 8.8 μmol/min, K_m = 236.7 mM), particularly at higher temperatures.

An amperometric cholesterol biosensor was created by immobilizing cholesterol oxidase onto electrospun polyaniline and polystyrene nanofibers (20). Polyaniline is a conductive material, allowing the cholesterol oxidase-modified fibers to be used as a working electrode. Polystyrene was used to enhance the spinnability of the polyaniline. The finished biosensor consisted of a nanofiber mat immersed in a beaker, with an Ag/AgCl reference electrode and platinum wire counter electrode. A glucose biosensor was also created through immobilization of glucose oxidase on a composite electrospun fiber composed of Prussian blue, chitosan, and PVA (21). Prussian blue is often used in amperometric glucose sensors due to its high biocompatibility and enhanced electron transport. However, in its native form it is not stable in neutral or alkaline solutions. Spinning the Prussian blue directly into the fibers resulted in improved stability in neutral and weakly alkaline solutions. The resulting sensor had a limit of detection of 3.61×10^{-7} M.

Antibodies

There are few reports on the successful spinning of antibodies into polymer-based nanofibers (8), which may be a result of the high costs of antibodies, limiting extensive spinning studies. However, the large surface areas of nanofiber mats have been utilized, primarily as scaffolds in tissue engineering, to immobilize antibodies after spinning (22).

Jin et al. (8) report a capillary flow microfluidic *Escherichia coli* biosensor that utilized water-soluble nanofibers for the storage of HRP-tagged antibodies. The HRP-tagged antibodies were incorporated into nanofibers composed of 15wt% PVP and 5wt% sucrose dissolved in distilled water. The spinning process dehydrated the antibodies and allowed them to be stored long-term in ambient conditions without losing functionality. These fibers were incorporated within microfluidic channels, and they dissolved upon contact with solution, releasing the antibodies. The three-dimensional structure of the nanofiber mats resulted in a uniform distribution of antibodies within the microfluidic channels. A limit of detection of 10^6 CFU/ml of *E. coli* O157 was reported, which demonstrated that the biofunctionality of the antibodies was retained after spinning and storage.

Electrospun PCL nanofibers have been used to create a matrix for the growth of endothelial cells (22). Anti-CD31 antibodies were immobilized on the scaffolds through adhesive proteins called hydrophobins, resulting in an increase in the attachment and retention of endothelial cells. The fibers were made of 10 w/v% PCL dissolved in dichloromethane (DCM) and dimethylformamide (DMF). Antibody immobilization did not require a cross-linking agent and took place using a self-assembled class II hydrophobin (HFBI) film on the electrospun fibers. After the HFBI film was deposited on the fibers, the fibers were functionalized by incubation in 10 μg/ml of primary antibody solution overnight at 4°C. Hou et al. (40) created a microfluidic chip for the detection, isolation, and analysis of circulating melanoma cells through the immobilization of anti-CD146 antibodies on poly(lactic-*co*-glycolic acid) (PLGA) nanofibers. Carboxylic acid groups on the PLGA fibers were activated using *N*-hydroxysuccinimide and used to covalently conjugate streptavidin to the fiber surfaces, which could then immobilize the anti-CD146 antibodies. Senecal et al. (41) report the development of carboxylated and aminated nanofibers for the covalent immobilization of antibodies. Carboxylated fibers were created by spinning polyvinyl chloride that was 1.8% carboxylated. Aminated functional membranes were made by spinning water-soluble polyamine and water-insoluble polyurethane. Antibodies could then be immobilized using conventional cross-linking chemistries.

Molecularly Imprinted Polymers

Molecularly imprinted polymers (MIPs) have been utilized to create synthetic, stable biological recognition elements for a variety of analytes. Several groups have investigated incorporating MIPs directly within electrospun nanofibers to make sensitive diagnostic devices and separation membranes (42–44).

Propranolol (PPL) MIPs were created from methyl methacrylate monomers, a PPL template, and a divinylbenzene cross-linker and then spun into 40 w/v% Eudragit-RS100 nanofiber mats (45). The resulting membranes were able to selectively bind PPL and did not bind other β-blockers such as atenolol, metaprolol, and timolol. Sueyoshi et al. (46) incorporated a MIP into electrospun chiral separation membranes. The membranes were composed of polysulfone with aldehyde (PSf-CHO-05 or PSf-CHO-10) and *N*-α-benzyloxycarbonyl-D-glutamic acid (Z-D-Glu) or *N*-α-benzyloxycarbonyl-L-glutamic acid (Z-L-Glu) serving as print molecules. A chiral selector composed from polysulfones was created for optical resolution (47). The membranes were able to purify target molecules from a solution containing both by-products of the target

molecule, solvents and contaminants. Nanofiber membranes prepared from polymeric materials had adsorption selectivity with mixtures of racemic Glu. The fluxes for the nanofiber membranes were increased two to three orders of magnitude when compared to traditional membranes (47).

Nucleic Acids

Aptamers are synthetic single-stranded nucleic acid sequences that can be used as biorecognition elements within biosensors due to their high affinity and specificity for target molecules (48). Typically, aptamers are used in protein purification systems by immobilizing the aptamers within monolithic capillary chromatography columns (49) or on magnetic or agarose beads within short-column systems (50, 51). Additionally, they have been incorporated in biosensors as an alternative to antibodies due to their relatively easy synthesis and high stability (52). Recently, several groups have utilized the large surface areas of electrospun nanofiber mats in the development of sensitive biosensors and protein purification scaffolds (51, 53, 54). Lee et al. (53) report the creation of a sandwich assay for the detection of thrombin by immobilizing two thrombin-binding aptamers, TBA29 and TBA15, on the surfaces of electrospun polystyrene-PSMA (PS-PSMA) nanofibers. The aptamers were immobilized on the nanofibers in a postspinning process by coating fibers with a solution of streptavidin and utilizing biotin-labeled TBA29 as the capture aptamer. Fluorescent detection of bound thrombin was achieved by using quantum dot or fluorescein dye-labeled TBA15. The resulting biosensor had a 25,000-fold higher sensitivity than a similar microwell plate assay, with a limit of detection of 10 pM with quantum dot detection. TBA29 immobilized on PS-PSMA nanofibers has also been used for purification of thrombin from solution (51). An 85% recovery yield of proteins was reported with the nanofiber-based purification system, which was both stable and reusable. The purification membrane was used repeatedly over a period of seven months and exhibited only a 10% reduction in recovery yield (51).

The traditional use of nucleic acids in hybridization with complementary DNA or RNA sequences has also been demonstrated. Specifically, a nucleic acid–based electrochemical biosensor for the detection of the *p53* tumor suppressor gene has been developed through electrospinning carboxylated multi-walled carbon nanotubes within a nylon 6 spinning dope (54). Electropolymerization was used to deposit pyrrole on the nanofiber surfaces, creating a composite nanofiber surface suited for immobilization of ssDNA and detection of the wild-type *p53* sequence. The resulting sensor had a limit of detection of 50 fm (54).

Biotin

Biotin is frequently utilized within sensors because of its rapid and specific binding with streptavidin (5). Generally, streptavidin is immobilized on the surface of a substrate and is then coated with a biotinylated biorecognition element to facilitate capture of target analytes (5). Nonwoven biotin-based fiber mats for use in biosensing platforms have been created by dispensing biotin in a PLA/chloroform/acetone spinning dope (5, 30). X-ray photoelectron spectroscopy revealed that there was a higher biotin concentration at the surface of the fibers than would be expected from uniform biotin distribution. A colorimetric assay demonstrated that the biotin retained its function and was able to bind streptavidin. Biotinylated nanofibers spun on copper-backed laminate were able to be incorporated within a lateral flow biosensor for the capture of *E. coli* DNA (5). Biotinylated poly(ethylene glycol)-b-poly(L-lactide)-b-poly(L-lysine) has been spun with poly(L-lactide-*co*-glycolide) into nanofibers to facilitate immobilization of proteins on nanofiber surfaces (55). The biotinylated nanofibers were successfully used to immobilize HRP-labeled streptavidin and rabbit anti-goat IgG.

Biotin has also been immobilized on nanofiber surfaces postspinning to allow for enhanced sensitivity within a biosensing platform (6). Wang et al. (6) electrospun poly(ethylene-*co*-glycidyl methacrylate) and cellulose acetate butyrate nanofibers that could be easily aminated and then biotinylated. HRP-labeled streptavidin could be immobilized on the fiber surfaces. The fibers were shown to be reusable, with 15% of relative activity retained after 10 repeated uses.

Pharmaceuticals

Several groups have developed biocompatible electrospun nanofibers encapsulating pharmaceuticals for localized drug delivery in vivo (56, 57). Utilizing drug-delivery scaffolds can improve delivery of pharmaceuticals to different tissue types, allow for lower doses than would be required for systemic drug delivery, and localize treatment only to affected areas (56, 58).

Antimicrobials and antibiotics can be incorporated within nanofiber mats to create dressings that deliver necessary pharmaceuticals directly to a wound (56). The goal is to decrease the bacterial load in a wound and simultaneously allow efficient drug delivery to tissues (such as granulating tissues) that do not receive sufficient antibiotics from system administration (56). The nanofiber mats can also be used to prevent postsurgical adhesions (58). PLGA has been utilized to create biodegradable nanofibers for antibiotic delivery in vivo (56). PLGA is a frequently used polymer that is fully biocompatible and can be tailored to maintain different degradation properties. It has been approved by the US Food and Drug Administration for use in numerous drug-delivery applications. Within the body, PLGA is degraded by hydrolysis and is metabolized by the citric acid cycle (56). The antibiotic cefazolin has been successfully spun within PLGA to create biodegradable wound dressings. This was done by dissolving both in a DMF and tetrahydrofuran solvent system (56). Cefazolin loading of 10% or 30% produced uniform fibers with good morphology. PLGA nanofibers have also been created for the sustained release of Mefoxin, a hydrophobic antibiotic (58). A 5w% drug loading percentage in the scaffold was investigated, which corresponds to one-twentieth of the daily systemic Mefoxin dose typically given after surgery. PLGA was used to control the degradation of the scaffolds, and PLA was utilized to create a mechanically strong scaffold. Finally, amphiphilic poly(ethylene glycol)-b-poly(lactide acid) (PEG-b-PLA) was also incorporated within the spinning dope to encapsulate the antibiotic. The final fibers were composed of PLGA:PLA:PEG-b-PLA in an 80:5:15 ratio. Drug release was sustained for one week and was effective in inhibiting *S. aureus* bacterial growth in agar and liquid environments. Hong et al. (59) created biodegradable poly(ester urethane) urea (PEUU) and PLGA nanofibers encapsulating tetracycline hydrochlorine through two-stream electrospinning. The PEUU provided increased elasticity to the scaffold, and the PLGA allowed for controlled drug release. The nanofibers were capable of sustaining drug release for one week in vivo and reduced abscess formation in rat abdominal surgery models (57, 60). Zeng et al. (57) created a poly(L-lactic acid) (PLLA) nanofiber containing rifampin, a tuberculosis drug, and paclitaxel, a chemotherapy drug. The spinning dope was composed of 3.9w% PLLA dissolved in a 2:1 (v/v) chloroform and acetone solution. Pharmaceuticals were placed directly into the spinning dope. The encapsulated pharmaceuticals were steadily released from the nanofibers through the action of proteinase K, with no burst release observed during the initial 7 h of use (57).

MIMICKING THE PHYSICAL STRUCTURE OF BIOLOGICAL MATERIALS

Biocompatible functionalized nanofibers are being investigated for use in cell culture or in vivo as tissue engineering scaffolds (61, 62). In these cases, the nanofiber mats are utilized for their

chemical functionality as well as their unique three-dimensional structures, which closely mimic the structure of the extracellular matrix (62, 63). Electrospun nanofiber mats are ideal for use as tissue engineering scaffolds due to their high porosity and very large surface areas, which make it easier to promote cell infiltration into the scaffolds (64, 65). Additionally, the mechanical properties of electrospun nanofibers can be tailored to mimic the properties of native materials. Although the materials presented here are not currently utilized in biosensors, their unique structures and functionalities make them ideal for use in sensors that monitor cell cultures or in vivo phenomenon and should be investigated for that purpose.

PCL is a biodegradable polymer commonly used in the creation of nanofiber-based tissue engineering scaffolds because it is nontoxic, inexpensive, and has slow degradation in cell culture and in vivo (61, 62, 65). Xu and coworkers (62, 63) created a nanofiber scaffold capable of mimicking the structure of the extracellular matrix from a poly(L-lactide-*co*-ε-caprolactone) [P(LLA-CL)] copolymer. The fibers had diameters between 400 and 800 nm, corresponding to the dimensions of the native extracellular matrix. When tested, the mechanical properties of the nanofiber mats were similar to those of a human coronary artery. The fibers were tested as a matrix for human smooth muscle cells as well as endothelial cells. Both cell types were seeded for seven days on the nanofiber mats and were capable of attaching and proliferating on the scaffolds. They retained their phenotypic shapes during growth and created a three-dimensional cellular network within the fibers. Choi et al. (61) have investigated the use of electrospun nanofibers made of PCL and collagen to create an implantable muscle tissue. The nanofiber mats were used to seed human skeletal muscle cells and their adhesion, proliferation, and organization were studied. Nanofibers that were unidirectional resulted in better muscle cell alignment and myotube formation, whereas randomly oriented nanofibers did not. Mesenchymal stem cells have also been cultured on PCL scaffolds to culture bone tissue (65). The stem cells were seeded on nonwoven PCL scaffolds created from a 10wt% PCL dissolved in chloroform spinning solution. After spinning, the mats were desiccated for several days, sterilized in a 70% ethanol solution, and then soaked in a collagen solution to allow for better cell attachment to the finished membrane. Histological studies of the nanofiber mats demonstrated that there was successful formation of extracellular matrix after one week and cells had migrated into the interior of the nanofiber mats. After four weeks, the extracellular matrix had an increased density, and significant calcification was observed. In addition, collagen type I was detected in the inner and outer portions of the nanofiber scaffolds after four weeks.

Poly(D,L-lactide-*co*-glycolide) (PDLGA) is another material frequently used as a scaffold in tissue engineering (66). Typically, three-dimensional PDLGA scaffolds can be created using a variety of methods, including molding/particulate leaching (66) or gas-forming and controlled-precipitation (67). Recently, there have been reports of electrospun PDLGA fiber mats used to create biocompatible scaffolds for in vivo use (68, 69). Xin et al. (69) examined whether human mesenchymal stem cells (hMSCs) grown on PDLGA nanofiber scaffolds could differentiate into chondrocytes and osteocytes. Fibers were created by dissolving PDLGA beads (85:15 PLA:PGA) in tetrahydrofuran and DMF. The PDLGA fibers were prewetted and sterilized with ethylene oxide prior to seeding of hMSCs. The scaffolds seeded with cells were cultured in a supplemented Dulbecco's modified Eagle's medium solution. Proliferation of the cells was observed, as indicated by an increase in the DNA contents of the hMSCs after 14 days of culturing. There was evidence that the PDLGA nanofibers were able to support the differentiation of hMSCs into osteoblasts and chondrocytes.

Natural polymers such as collagen and elastin have also been utilized to create highly biocompatible nanofiber mat scaffolds (64). As previously discussed, nanofiber mats made from PCL are sometimes soaked in collagen solutions prior to use in order to facilitate cell adhesion (65). Incorporating collagen or elastin directly in the spinning dope simplifies the fabrication process

and results in a nanofiber mat that has enhanced cell adhesion properties. In particular, collagen and elastin nanofibers have been shown to promote infiltration of cells into the scaffold mats and are able to supply cells with oxygen and nutrients (64). However, when these materials are spun without the support of a synthetic polymer, mats show dimensional instability, resulting in early dissolution of fibers in culture as well as shrinkage of the mats (64). Lee et al. (64) created blood vessel scaffolds from electrospun nanofibers consisting of biodegradable polymers such as collagen and elastin. Fibers created from a 45w% collagen, 15w% elastin, and 40% synthetic polymer solution [PDLGA, poly(L-lactide), poly(ε-caprolactone), and poly(D,L-lactide-*co*-ε-caprolactone)] were able to mimic the collagen and elastin composition of blood vessels. The scaffolds utilized randomly oriented fibers and proved nontoxic and stable in in vitro cultures. Furthermore, the mechanical properties of the mats were able to simulate the structure of nanotube blood vessels and were simultaneously easy to fabricate.

Heterogeneous nanofiber mats have also been developed to more closely mimic the structure of biological tissues. Xie et al. (23) utilized PCL dissolved in DCM and *N*,*N*-dimethylformamide at a ratio of 4:1 v/v at 10% w/v concentration to create nanofiber scaffolds with gradations in the fiber organization. Two nanofiber types, one composed of PCL and the other PCL and coumarin 6 dye, were spun from different syringes. The resulting composite fiber mat had a chemical gradient from spinning the second mat of coumarin fibers directly on top of the PCL fibers, resulting in the deposition of random fibers on uniaxial aligned fibers. Ionescu et al. (70) have reported the creation of chemically heterogeneous cell culture scaffolds through cospinning PEO fibers encapsulating PLGA microspheres and mechanically strong PCL nanofibers. The PEO fibers served as a sacrificial membrane and degraded upon contact with solution, leaving behind a stable PCL fiber matrix interspersed with a high concentration of PLGA microspheres. Both BSA and chondroitin sulfate were encapsulated in the PLGA microspheres, resulting in nanofiber scaffolds capable of sustaining a dual-release profile for 35 days (70). The authors hypothesize that more complex chemical gradients, such as growth factor cascades, could be simulated through controlled encapsulation of different biomolecules within the PLGA microspheres (70).

POSSIBLE APPLICATIONS: THE FUTURE OF FUNCTIONALIZED NANOFIBERS IN BIOANALYTICAL SYSTEMS

Electrospun nanofibers are frequently utilized in many biological fields, including tissue engineering (68), drug delivery (60), and to a very limited degree, biosensing (21, 34). In biosensing, functionalized nanofibers have primarily been used to increase the number of binding sites available, resulting in better immobilization efficiencies and detection sensitivities (4). However, the full potential of these nanoscale materials to significantly enhance the performance of bioanalytical devices has yet to be realized. We predict that utilizing the many exciting surface chemistries and structures offered by electrospun nanofibers within both simple lateral flow assays and complex lab-on-a-chip systems could revolutionize the way biosensing is done today. For example, the functionalized nanofibers presented in this review could be used to create highly sophisticated integrated sensing systems, particularly for cell culture and in vivo sensing applications (**Table 3**).

Our research group's experience with the integration of nanofibers within bioanalytical platforms demonstrates their general applicability for sensing and sample preparation in lateral flow and microfluidic devices (5, 13, 71). Specifically, we have integrated charged PVA nanofibers into microfluidic channels to serve as bioseparators. The fibers spun from a PVA-polybrene spinning dope have successfully been used to capture and release liposomes by controlling the pH of the solution within a microfluidic channel (13) (**Figure 2**). We have also developed de novo nanofiber mats as membrane materials in lateral flow assays (5, 30, 71) (**Figure 2**). These

Table 3 Nanofibers used within other fields that have direct applications within biosensing

Polymer	Biological additive	Functionalization method	Results	Citation	Potential bioanalytical use
PCL	Anti-CD31 antibodies	Postspinning immobilization using adhesive proteins (hydrophobins)	Increased attachment and retention of endothelial cells	22	Enhanced biorecognition element immobilization
	Collagen	Co-electrospinning	Muscle cell culture and myotube formation	61	Biocompatible fibers for in vivo or cell culture sensors
	Coumarin (dye)	Incorporation in spinning dope	Heterogeneous nanofiber mats	23	Bioanalysis of effects of chemical gradients on cell cultures
PLGA	Anti-CD146 antibodies	Postspinning covalent conjugation of streptavidin on fiber surfaces	Isolation of circulating melanoma cells	40	Generation of generic immobilization surfaces
	Cefazolin (antibiotic)	Incorporation in spinning dope	Drug-eluting wound dressing	56	Sensor to test toxicity of drugs within cell cultures
PVP	Anti-*E. coli* antibodies	Incorporation in spinning dope	Improved antibody storage in ambient conditions	8	Stable storage of reagents and biorecognition elements in sensors

Abbreviations: PCL, poly(ε-caprolactone); PLGA, poly(lactic-*co*-glycolic acid); PVP, polyvinylpyrrolidone.

nanofiber mats can be used for antibody immobilization, development of standard sandwich assays using antibodies and DNA oligonucleotides as biorecognition elements, and prevention of any nonspecific binding by incorporation of polystyrene$_{8K}$-*block*-poly(ethylene-*ran*-butylene)$_{25K}$-*block*-polyisoprene$_{10K}$-Brij76 (KB) into PLA-PEG nanofibers (71).

On the basis of our experience and considering the superior performance of nanofibers in tissue engineering and drug-delivery applications, we provide below several concrete examples and predictions of how electrospun nanofibers published and used in other disciplines could be integrated into biosensor platforms and improve specific sensing capabilities.

Improving Sensor Performance

There are many advantages to nanofiber-based sensing platforms other than an increased surface area and detection sensitivity. Several groups have demonstrated that immobilization of biological molecules directly within fiber spinning dopes can result in better long-term stability in ambient and harsh conditions (8, 21, 38). This is crucial for the development of biosensors for use in point-of-care settings, where refrigeration may not be available. Particularly useful is the notion of encapsulating reagents and biorecognition elements directly within water-soluble fibers for long-term storage in microfluidic sensors (8, 38). Furthermore, enhanced stability of enzymes within electrospun nanofibers allows for the development of sensors that can function over a wider range of temperatures and pH values (12, 38).

Biosensors that utilize BSA or casein-based nanofibers could be used to significantly reduce nonspecific binding (9, 15). Because nanoscale materials have been incorporated within globular protein-based nanofibers, it would be possible to create a sensing platform that allows for specific detection of an analyte through incorporation of an antibody or enzyme within the nanofiber with simultaneous prevention of nonspecific binding by utilizing a BSA or casein nanofiber base. This would eliminate or reduce the need for additional blocking steps within the device, resulting in

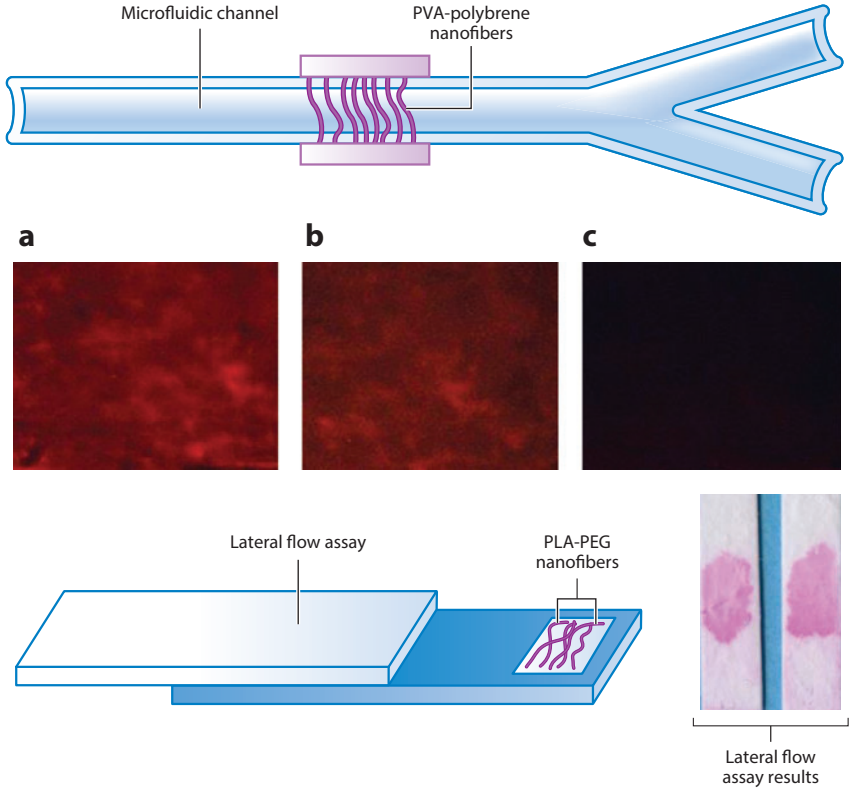


Figure 2

(*top*) Microfluidic channels containing PVA-polybrene nanofibers. (*a*) They were first filled with fluorescent liposomes at pH 7. (*b*) Liposomes remained on the nanofiber mats after 20 min of washing with a pH 7 buffer solution. (*c*) Bound liposomes were then eluted from the mats using a pH 9 washing step (13). (*bottom*) Nanofibers functionalized with anti-*Escherichia coli* antibodies were incorporated into lateral flow assays for the detection of *E. coli* 0157 (71). Abbreviations: PEG, poly(ethylene glycol); PLA, poly(lactide); PVA, poly(vinyl alcohol).

more streamlined biosensors. Furthermore, it would also avoid the negative side effect of blocking binding sites, which occurs in bulk blocking procedures.

Multiplexed detection is also possible through the incorporation of more than one type of functionalized nanofiber within a biosensor. Heterogeneous nanofiber mats have been created through the cospinning of two different polymer spinning dopes, and the same technology could easily be applied for the creation of biosensing platforms (23). Additionally, the principle of coaxial spinning can be utilized to incorporate two functional materials within the same nanofiber, with one material concentrated at the core and one at the shell of the fiber. This has already been successfully demonstrated in the development of tissue engineering scaffolds that utilize a biodegradable shell to control the release of proteins from the core of the fiber (16). However, it could be used easily to create a biodegradable fiber that releases two functional molecules over time to facilitate multiplexed detection. Finally, the microsphere-encapsulating nanofibers reported by Ionescu et al. (70) could be used to release multiple biorecognition elements into a sample matrix, allowing for the detection of many target analytes.

Cell Culture Sensors

Biosensors have been incorporated within cell culture platforms to assess the response of different cell types to pharmaceuticals, nanoparticles, and growth conditions (72, 73). In general, three-dimensional cell culture scaffolds better replicate in vivo cellular conditions when compared to 2D cultures composed of monolayers (72, 74). As described above, due to their highly porous three-dimensional structure, nanofiber scaffolds can support cell growth, morphogenesis, cell metabolism, and cell-to-cell communication. Additionally, because electrospun nanofibers can incorporate a variety of functional additives, there is the potential for the development of nanofiber scaffolds that couple cell culture with direct monitoring of cell response to stimuli. For example, the highly biocompatible BSA nanofibers produced by Valmikinathan et al. (33) can be functionalized with FITC to produce a noninvasive pH sensor for use within cell culture platforms. Additionally, enzymes could be incorporated within nanofiber scaffolds to allow for real-time analysis of cell metabolism and toxicity.

One of the main applications of cell culture biosensors is testing the toxicity of pharmaceuticals on different cell types (73, 74). As discussed, nanofibers capable of eluting pharmaceuticals have been developed and could be incorporated directly within cell cultures to facilitate analysis. The release of pharmaceuticals into cell culture could be regulated by controlling the specific composition of nanofibers used. In addition, the large surface area and three-dimensional structure of the nanofiber mats could help ensure that there is a uniform distribution of the drug within the cell culture, eliminating inconsistencies in cellular dosing and response.

Another application of cell culture biosensors is monitoring the metabolism of different cell types (75). Integrating nanofibers containing glucose oxidase directly within cell culture scaffolds could facilitate real-time monitoring of cellular metabolism (21). Additionally, incorporation of conductive nanofibers that have been rendered biocompatible through coating or cospinning with biocompatible polymers could facilitate electrochemical detection (27).

In Vivo Sensors

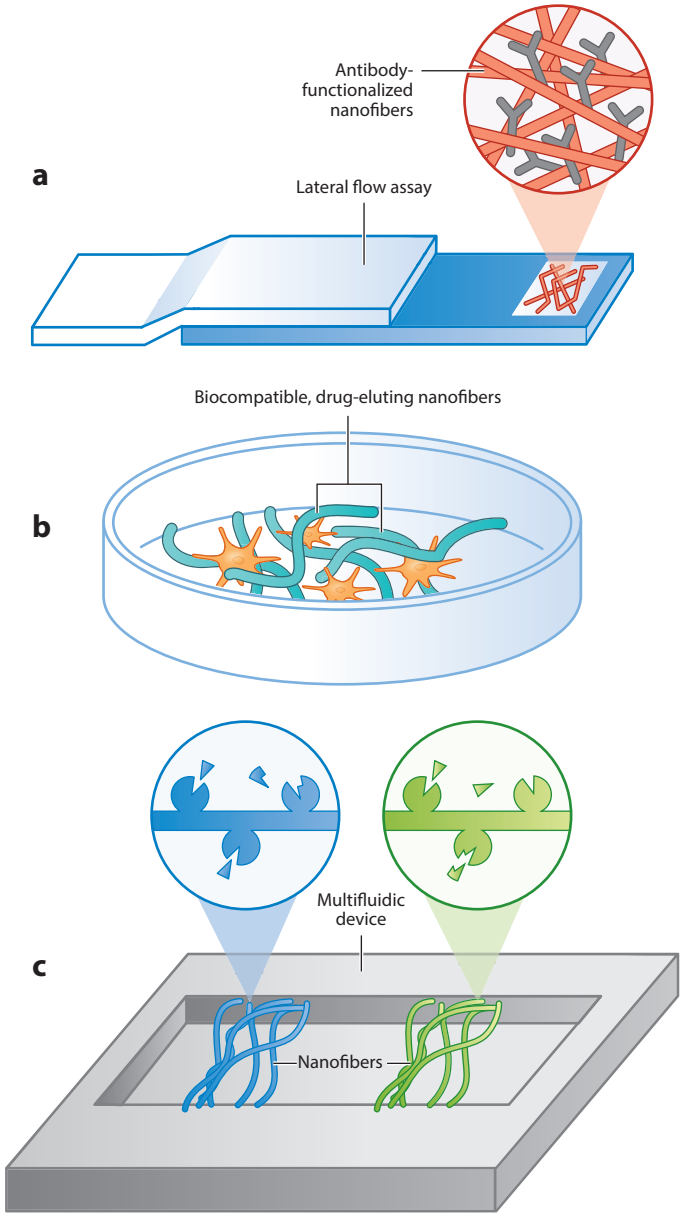
In vivo biosensors have the potential for allowing continuous, real-time monitoring of a patient. For example, because of the feasibility of developing them, subcutaneous continuous glucose sensors have been investigated as a desirable alternative to conventional glucose monitoring (76, 77). However, the development of in vivo sensors is complicated by their many requirements. Generally, in vivo sensors need to be reagentless, stable in hostile environments, and biocompatible (76–78). Most importantly, the sensor must have a very limited effect on surrounding tissues to ensure that it is not rejected and that sensor results are not altered by a nearby inflammatory response. Several approaches have been investigated in the creation of in vivo sensors, including batch sampling of biological fluids, indwelling catheters, microdialysis, and subcutaneous implants (76). Electrospun nanofibers, functionalized directly with the necessary enzymes and sensing elements, could address the need for reagentless detection and simultaneously increase the biocompatibility of the implant. Furthermore, biodegradable fibers that elute antibiotics or antimicrobial agents could be used to reduce inflammation and reaction around implant surfaces.

CONCLUSION

Electrospun nanofibers are a versatile material with high surface-area-to-volume ratios, large porosity, and a wide range of available surface chemistries (14, 28). They have been used with great success in tissue engineering (18, 62, 67) and drug-delivery platforms (56, 57), and are

Figure 3

Future applications of functionalized nanofibers in biosensing. (*a*) Antibody-functionalized nanofibers incorporated into lateral flow assays to increase limit of detection, improve device stability in ambient conditions, and simplify immobilization. (*b*) Cell culture sensor created from biocompatible, drug-eluting nanofibers. (*c*) Multiplexed sensing facilitated by spinning multiple types of nanofibers in microfluidic devices.



increasingly being utilized in sensing platforms (8, 9, 21). Generally, nanofiber-based biosensors have improved sensitivity due to the increased immobilization efficiency of biorecognition elements (4). However, nanofibers offer much more than immense surface areas, as demonstrated by the many useful surface chemistries described in this review. Consequently, it is time to translate knowledge of nanofiber composition and fabrication generated from other disciplines into novel, improved bioanalytical systems (**Figure 3**). In particular, electrospun nanofibers can be used to immobilize biorecognition molecules, create nonspecific binding-resistant surfaces, increase stability of biological molecules, and provide orientation of biorecognition

elements. Nanofibers should therefore be utilized within bioanalytical systems to enhance sample preparation (filtration, separation, and concentration), binding events, and signal transduction. Miniaturized bioanalytical systems can particularly benefit from the use of electrospun nanofibers to enable further development of portable devices that require smaller reagent and sample volumes than traditional devices, making them more accessible for use in point-of-care settings (1, 4).

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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