Electrospun composite nanofibers as sensors for food analysis

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Rafaela S. Andre[a], *Luiza A. Mercante*[a], *Murilo H.M. Facure*[a,b], *Adriana Pavinatto*[c], *and Daniel S. Correa*[a,b]

[a]Nanotechnology National Laboratory for Agriculture (LNNA), Embrapa Instrumentação, São Carlos, SP, Brazil, [b]PPGQ, Department of Chemistry, Center for Exact Sciences and Technology, Federal University of São Carlos (UFSCar), São Carlos, SP, Brazil, [c]Scientific and Technological Institute of Brazil University, Universidade Brasil, São Paulo, SP, Brazil

8.1 Introduction to polymer-based nanofibrous composite materials

Over the past decade, the prodigious development of nanotechnology and nanoscience has allowed the fabrication of high-performance devices aimed at different areas of applications such as solar-cells, data-storage devices, and light-emitting diodes and sensors [1–4]. For instance, the application of nanotechnology in the food industry sector has led to the development of reliable sensors capable of monitoring food quality and freshness, which can help avoiding food contamination or waste [5–8]. Distinct materials have been used for the design of high-performance sensors, including conducting polymers and their composites [9–11]. Conducting polymers possess π-conjugated bonds along the polymer chain, and such electron delocalization leads to inherent optical and electrical/electronic properties similar to some metals and semiconducting materials [12]. Besides, conducting polymers present the advantages of chemical diversity, flexibility, corrosion resistance, easy-to-control shape and morphology, and tunable conductivity [13]. Such outstanding properties allow the development of high-performance sensors or even flexible and wearable devices [14]. Polypyrrole (PPy), polyaniline (PANI), and poly(3,4-ethylenedioxythiophene) (PEDOT) are the most investigated conducting polymers for sensors devices. For example, PANI has been employed in optical-chemical sensors to detect both alcohol and chloroform, depending on the dopant agent and ratio, demonstrating its outstanding potential and easily modulated performance [15, 16].

Despite the appealing features of conducting polymer-based devices, smart devices require distinct properties (e.g., flexibility, fast response, high sensitivity, low detection limit, and reproducibility combined to low cost) that cannot be fulfilled using only one class of material. Such combination of properties can be achieved by designing composites through the use of dissimilar materials such as polymers, metal oxides (ZnO, TiO_2, SnO_2, WO_3, among others), metallic nanoparticles (Au, Ag, Pd, and Cu), and chalcogenides (CdSe, ZnS, CdTe among others) [17–19]. In general, composites are multiphase materials obtained with chemically dissimilar constituents

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called the matrix (the constituent in higher concentration) and filler (the constituent in lower concentration). More recently, nanocomposites, which are composite materials having at least one of the phases at the nanoscale, have been designed for high-performance applications [20–22]. Nanocomposites can be classified into two major categories namely polymer-based and nonpolymer-based nanocomposites. However, studies involving new technologies and smart devices significantly focus on polymer-based nanocomposites due to their superior performance [23]. Usually, they are prepared by adding inorganic nanoparticles (ceramic and metallic) or carbon-based nanomaterials (graphene, carbon nanotubes, and carbon quantum dots) to the polymer matrix in order to enhance their final properties. Nanocomposite's properties depend on the nature, morphology, and other properties of the individual constituents as well as on the interfacial adhesion and fraction between matrix and filler. The optimized properties achieved by nanocomposites enable them to be employed in various applications, from high-performance mechanical devices to sensors and biosensors.

Chemical sensors are generally composed of an ultrathin sensing layer deposited onto a substrate integrated with the transductor. Depending on its chemical composition, the sensing layer can interact with varied analytes, allowing their identification and quantification through changes in electrical, electrochemical, or optical properties of the system under investigation. In this context, nanofibers (NFs) have been proposed as a potential candidate to be used as an active layer of high-performance and flexible chemical sensors [24–26]. Due to their high-specific surface area and interconnected porous structures, NFs are able to create a 1D conducting channel at a short range and a 3D web-like structure in a long range. Such features can enhance the active layer response toward analytes detection. Besides, NFs can be obtained from a wide range of materials to produce composite materials. For instance, composite nanofibers can be obtained through electrospinning using an insulating matrix and a conducting "filler" of varied composition, which can enhance the sensor performance [21, 22].

The appealing features of composite NFs (depicted in Fig. 8.1A) make them highly useful for fabricating chemical sensors and biosensors [27]. Fig. 8.1B shows the

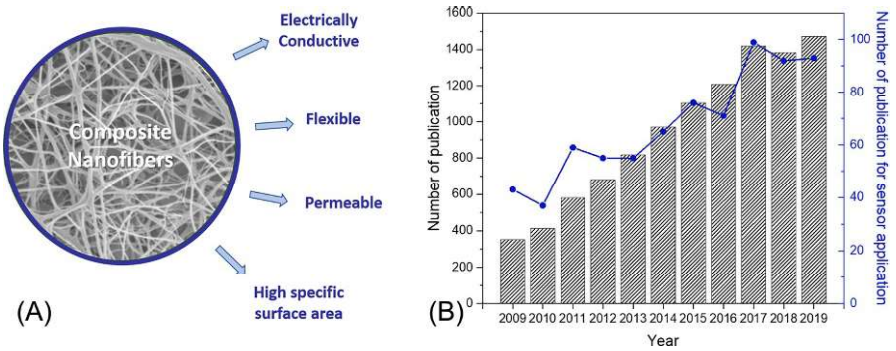

Fig. 8.1 (A) Composite nanofibers advantageous features and (B) the number of scientific publications per year related to composite nanofibers (*bar chart*—left *y*-axis) and those regarding sensor application (*scatter line*—right *y*-axis) for the period 2009–19 (data retrieved from Web of Science in May 2020).

number of publications in the past 10 years dealing with electrospun composite NFs and those applied in sensors, evidencing the scientific and technological relevance of this type of material. In this context, this chapter reviews different composite NFs that have been used in varied sensors for monitoring food and beverage quality regarding microbial and nonmicrobial contamination. Fiber fabrication methodologies are presented and discussed in detail aiming to contribute and encourage the development of future works with composite NFs for high-performance devices.

8.2 Nanofiber fabrication techniques

8.2.1 Electrospinning

Of all the strategies available for producing ultrafine fibers [28, 29], electrospinning is one of the most established and widely adopted methods. Electrospinning has been successfully used to produce NFs with diameters down to tens of nanometers, using a huge variety of materials, including polymers, ceramics, and their combinations [30]. The electrospinning technique was first developed and patented by Formhals in the 1930s [31] and later developed by Reneker and coworkers [32]. Since then, a huge number of investigations have been consistently reported. According to Web of Science (data obtained in May 2020), more than 28,000 scientific publications and patents on electrospinning have been published.

A typical electrospinning setup consists of three essential parts: a spinneret, a ground collector to collect the nanofibers, and a high-voltage power supply to generate a strong electric field between the spinneret and the collector, as illustrated in Fig. 8.2A. In general, the fiber formation through the electrospinning process can be divided into three consecutive steps: (1) onset and developmentof solution jetting; (2) bending deformation with looping and spiraling trajectories, and nanofiber solidification with the evaporation of solvents; and (3) nanofibers collection [33, 34]. Initially, when a small amount of a viscoelastic fluid is pumped out through the spinneret, it tends to form a spherical droplet as a result of the confinement of surface tension (Fig. 8.2B). Since the droplet is subject to an electrical field applied by the high-voltage source, its surface will be quickly covered by charges of the same sign [35]. The repulsion among these charges will counteract the surface tension and destabilize the spherical shape elongating the droplet into a conical shape known as a Taylor cone [36], as shown in Fig. 8.2B. At a critical point, when the electric force is strong enough to overcome the surface tension force, a tiny jet of the polymer solution is drawn from the tip of the Taylor cone, resulting in the ejection of the polymer solution jet [37]. The traveling liquid jet stream is subjected to several opposing forces, resulting in fluid instabilities [37], leading to the whipping motion of the jet between the capillary tip and the collector. This process is responsible for thinning the initial jet into a nanometer fiber. At the same time, the solvent is evaporated, and the nanofibers are collected in the form of nonwoven webs [38].

In spite of the simple setup, the properties of electrospun fibers can be greatly affected by a number of parameters, including: (1) the intrinsic properties of the solution, such as the type of polymer, concentration, conductivity, and solvent volatility;

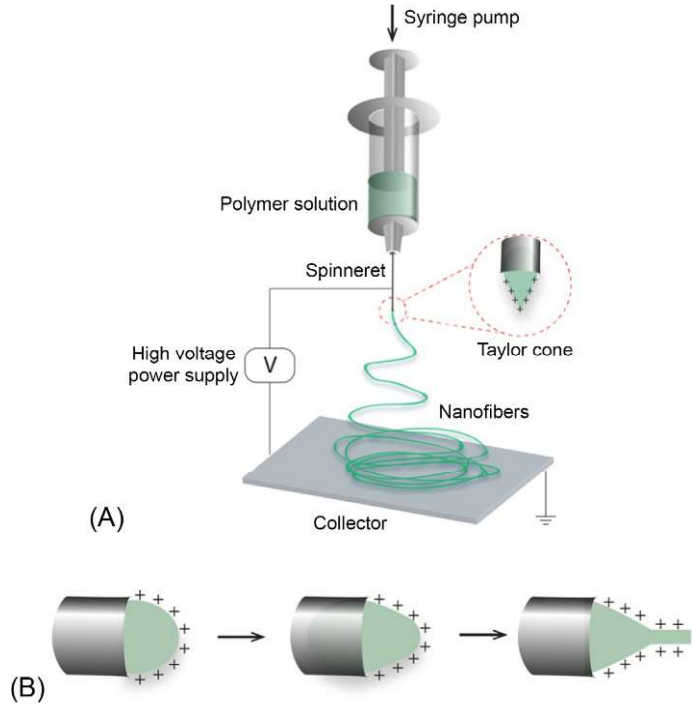

Fig. 8.2 (A) Schematic diagram of a typical electrospinning setup comprising of three components: a conductive spinneret to infuse the polymer solution, a grounded conductive collector, and a high-voltage power supply. (B) Formation of the Taylor cone under the electric field. When the electric force that pulls the polymer solution downstream is strong enough to overcome the surface tension that holds the polymer solution to the tip, a tiny jet of the polymer solution is drawn from the tip of the Taylor cone and its solvent evaporates quickly, forming solidified polymer fibers.
Adapted and reprinted with permission from L.A. Mercante, V.P. Scagion, F.L. Migliorini, L.H.C. Mattoso, D.S. Correa, Electrospinning-based (bio)sensors for food and agricultural applications: a review, Trends Anal. Chem. 91 (2017b) 91–103. doi:https://doi.org/10.1016/j.trac.2017.04.004 (web archive link). Copyright 2017 Elsevier.

(2) the processing parameters such as the strength of the applied electric field, solution flow rate, and the distance between spinneret and collector; and (3) the humidity and temperature of the environment [39]. Each of these parameters can affect the morphology of the fibers obtained as a result of electrospinning. However, by proper manipulation of the parameters, it is possible to obtain nanofibers of desired morphology and diameters [31].

Additionally, the method is versatile and adaptable. Recently, several innovative electrospinning processing approaches such as needleless electrospinning [40], melt electrospinning [41], coaxial electrospinning [42], emulsion electrospinning [43], and co-electrospinning [44] have been developed to provide a wide variety of nanoarchitectures [35].

The multineedle and needleless electrospinning have been applied to enhance the productivity of the conventional method [40]. The fundamental principle to obtain higher productivity is based on increasing the number of jets by adopting different configurations, including multijets from the single needle and multijets from multiple needles and needleless systems [41]. On the other hand, coaxial electrospinning was developed to produce core-shell and multilayer composite structures with additional functionalities and improved quality [29]. In this process, two different polymer solutions are supplied through different coaxial capillary channels and then integrated into core-shell nanofibers. The development of coaxial electrospinning has significantly contributed to the development of numerous novel composite nanomaterials with advanced sensor performance [45]. For example, Huang and coworkers reported the fabrication of In_2O_3 NFs decorated with Au nanoparticle by coaxial electrospinning for efficient sensing of ethanol gas at low temperatures. The composite nanofiber exhibited a higher response and a shorter response/recovery time to 100 ppm ethanol gas when compared to the pristine In_2O_3 nanofiber [46]. In addition, by removing the core material via solvent dissolution or calcination, hollow structured polymeric or ceramic nanotubes can be obtained [47].

Moreover, side-by-side dual-capillary spinneret has been designed to prepare bi-component nanofibers. Using such a route, a range of polymeric and ceramic nanofibers with Janus structures have been fabricated [47, 48]. The structural asymmetry of Janus architecture that contains compartments with different chemical, optical, electrical, or magnetic properties (which are incompatible when they are combined on the same surface) can improve the sensing performance in a single structural unit [49, 50]. For instance, Janus nanofiber array with magnetic-fluorescent bifunctionality was successfully prepared by Wang et al. [48]. Compared with the counterparts, the Janus nanostructure presented the highest fluorescence intensity owing to its peculiar structure, showing potential for chemical sensing applications. The exploration of electrospinning for the creation of complicated nanostructures is still growing and these findings have great potential in the development of advanced materials.

8.2.2 Solution blow spinning

An alternative method to produce nonwoven polymer mats, called solution blow spinning (SB-spinning), has also become a potential candidate to fabricate ultrafine materials. This method has gained popularity among researchers, despite its recent advent in the early 2000s [32, 51, 52, 110], including for applications in chemical sensors [53–55]. This technique has been developed to overcome some drawbacks of the electrospinning technique, such as the difficulty to allow in situ synthesis of nanofibers, low-output production, and the requirements for high electrical potential and conducting targets [51]. SB-spinning apparatus is comprised of concentric nozzles and a source of compressed air that works as the driving force for micro- and nanofiber formation. The spinning solution is injected through the inner nozzle, while a pressurized air (generally of the order of 150–200 m/s at 3–5 bar of pressure)

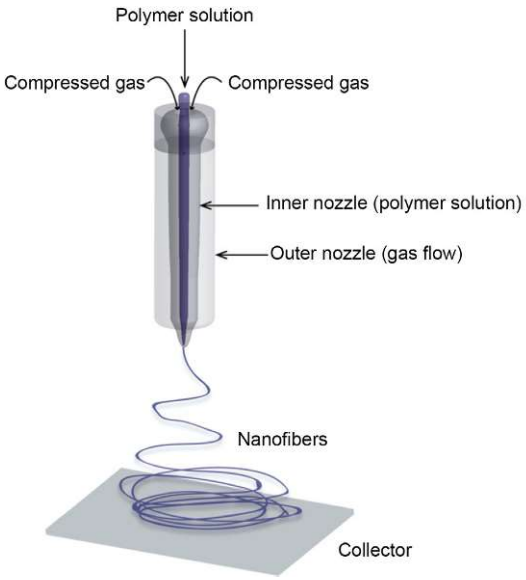

Fig. 8.3 Schematic representation of a solution blow spinning device showing concentric nozzles. Adapted and reprinted with permission from L.A. Mercante, M.H.M. Facure, D.A. Locilento, R.C. Sanfelice, F.L. Migliorini, L.H.C. Mattoso, D.S. Correa, Solution blow spun PMMA nanofibers wrapped with reduced graphene oxide as an efficient dye adsorbent, New J. Chem., 41 (17) (2017a) 9087–9094. https://doi.org/10.1039/C7NJ01703K (web archive link). Copyright 2017 Royal Chemical Society.

[32] is released through the outer nozzle, as illustrated in Fig. 8.3. The pressurized air at the tip of the inner nozzle then stretches the polymer solution. After a critical pressure is exceeded, a jet flows from the stretched tip toward the collector. During jetting, the solvent evaporates and a solid polymer fiber forms which can be collected on a target. SB-spinning has production rate that is about 100 times or higher than that reached for electrospinning, combined to at an overall lower cost [52].

Since SB-spinning is a much younger technique, studies dealing with the prediction and control of fiber diameter and morphology are still scarce [56]. However, some studies have already shown that the solution flow rate, the distance from the nozzle to the collector, and the protrusion length of the inner nozzle are some important parameters in this technique [57]. Additionally, the polymer type and concentration also affect the fiber morphology. For instance, Khattab and coworkers demonstrated an increase in fiber diameter with the increase of polymer solution concentration when the solution blowing spinning technique was employed to develop the nanofibrous colorimetric sensor [53]. The gas flow pressure has also influence on the fiber morphology. Generally, increasing gas pressure produces a narrower fiber diameter distribution, which is preferred for sensing applications that require precise fiber diameter [51]. Nevertheless, increasing the gas flow rate beyond the optimal range may also cause a temperature decrease due to gas expansion, which may cause poor solvent evaporation and promotes instability during the production of the fiber, yielding to fiber coalescence [58].

8.2.3 Recent strategies for nanofiber fabrication

Although most nanofibers are prepared using electrospinning or solution blow spinning techniques, these methods present some drawbacks, as illustrated in Table 8.1. Therefore, other approaches have been developed to improve fiber production [29]. Some of these methods include drawing techniques, template synthesis, centrifugal spinning, phase inversion/separation, freeze/drying synthesis, interfacial polymerization, and self-assembly [57]. Among these, centrifugal spinning might be seen as the most advantageous from the aspect of industrialization and scalability [60]. In this approach, centrifugal forces are used to initiate jet formation, unlike electrospinning and solution-blowing techniques. Three main stages take place during the fiber formation process: jet initiation, jet elongation, and fiber formation/solvent evaporation. In general, a polymer solution or melt is fed into a rotating chamber, consisting of two or more orifices. During the rotation, the centrifugal force pushes the polymer solution/melt toward the inner surfaces of the chamber, guiding it toward the orifices. When the centrifugal force exceeds the surface tension and the viscosity of the solution/melt, the polymer jet exits from the orifices elongates, and the solvent evaporates until the polymer fiber reaches the collector plane [57]. One of the most notable advantages of the centrifugal jet spinning technique lies in its exceptionally high production rate. Specifically, its productivity is estimated to be approximately 500 times higher than that of conventional electrospinning [29]. Centrifugal spinning has been recently applied by Xu et al. as an efficient and rapid method to fabricate ultrafine fibers for sensing applications [61]. In their work, the main parameters of the centrifugal spinning process such as solution concentration, rotational speed, and spinneret diameter have been investigated for the synthesis of SnO_2 NFs aimed at gas sensing. The SnO_2 sensor showed the highest sensitivity for HCl and the sensitivity for ethanol gas increased when its concentration exceeded 1200 ppm.

Table 8.1 Advantages and disadvantages of electrospinning and solution blow spinning techniques.

Nanofiber fabrication method	Advantages	Disadvantages	References
Electrospinning	Vast material selection Easy to incorporate additives in nanofibers Practicability in generating nanostructures, including core-sheath, Janus, and tri-layer nanofibers	Low production rates High electrical voltage required to produce the nanofiber	[29, 59]
Solution blow spinning	High production rate In situ production Free from high voltage	Variable diameters distribution	[29, 40]

8.3 Electrospun composite nanofibers for designing food sensors

Food contamination represents a severe public health concern around the world and a serious problem for the food industry. Safe food should not present any harm to health or cause illness or injury [62, 63]. A contaminated product accidentally ingested by humans can lead to a variety of foodborne diseases [64]. Contamination of food during any stage of the processing chain can lead to enormous economic loss for the industry [65]. Although many situations can cause food contamination, viz., during or after its production, processing, packaging, transportation, storage, and consuming process, most of them can be classified as biological, chemical, physical, and cross-contamination [66]. In this scenario, the use of accurate analyses can be crucial to avoid health problems. Additionally, the current level of globalization, the high-speed demanded by an increasingly competitive market and the ever-changing and diversified food laws and regulations imposed by governments require robust, sensitive, fast, and low-cost food analyses [67, 68]. In the next sections, we will discuss on some types of sensors based on electrospun NFs aiming at detecting nonbiological and biological contamination in food samples.

8.3.1 Sensors for detecting nonmicrobial contaminants in food samples

"Food" refers to any processed, semiprocessed, or unprocessed item that is intended to be used as food or drink, therefore encompassing a huge variety of products. The components that may impair the quality of the food can be biological contaminants, such as pathogenic bacteria, parasites, and viruses (discussed in more details in the next section) or chemical contaminants, e.g., heavy metals, pesticides, drugs, and hormones, coming from agricultural residues, additives, industrial activities, or even naturally occurring harmful compounds [62, 68]. Despite a large number of available detection methods, choosing the appropriate alternative relies on the kind of food that will be analyzed and the hazardous components to be investigated [67]. Accordingly, the correct analytical choice must represent a reliable methodology that fits properly in specific situations, giving rise to complete and accurate information [69].

An alternative to achieve a fast and robust response is through the use of sensors in food analyses. They represent a promising tool since they can be employed in the determination of food composition, nutritional information, and degree of contamination, and in the identification of undesired and harmful compounds. Stable devices with high sensitivity and selectivity, reproducibility, fast response time and the possibility to be reused have been proposed for monitoring food quality and safety [63, 70]. The sensor acts by transforming chemical information, i.e., changes occurring between the analyte and the sensitive layer, into an analytically measurable and useful signal that will be processed and interpreted using pattern recognition methods [9].

Aiming to improve the sensing performance and reach miniaturized devices, nanofibers arise as an interesting nanostructure to be used as a constituent of the

sensing material. Their characteristics, such as high porosity and high specific surface area, increase the ability of the sensing material to interact with the medium, enabling the achievement of enhanced sensing capabilities [39]. Furthermore, electrospun NFs can be obtained from different raw materials and can be functionalized with a wide variety of nanomaterials exhibiting distinct interacting properties to be tailored according to the final application [58, 71].

In such scenario, the electronic tongue (e-tongue) stands out as a remarkable instrument that can perform analyses of food quality [5, 72, 73], making possible the detection of food adulteration, authenticity, and hazardous residues. Additionally, such an approach also presents the advantages of being a nondestructive simple, sensitive, low-cost, and fast method. In addition to being useful for detecting contaminants that may present risks to humans, the e-tongue can reach levels of detection much inferior to those of human capacity [74]. The e-tongue system is usually comprised of three parts: an array of nonspecific sensors, a signal acquisition system, and a pattern recognition method employed for data treatment [75]. The response of all sensing units together should be able to respond to different analytes in solution without (or with low) specific interaction. In this manner, the composition of simple and complex solutions can be quantitatively and qualitatively analyzed, providing information that allows the identification, classification, and discrimination of the samples under analysis [76].

A crucial step to achieve the best performance of an e-tongue lies in the choice of the material that will compose the active layer of the sensing units. For instance, Scagion et al. developed an e-tongue composed of conducting electrospun NFs in order to detect antibiotics in milk samples [77]. Specifically, electrospun polyamide 6 (PA6) and polyamide 6/polyaniline (PA6/PANI) nanofibers were deposited onto gold interdigitated electrodes (IDEs) to compose the array of sensors used in an impedimetric e-tongue. PANI was chosen because of its high electrical conductivity, while PA6 was used to facilitate the electrospinning process. The system was used to identify the presence of residues of tetracycline (TC), a class of broad-spectrum and effective antibiotics, in fat and skimmed milk samples. Traces of this sort of contaminants can lead to losses in fermented products. Therefore, samples of contaminated milk were prepared in concentrations ranging from 300 ppb down to 1 ppb of TC. The electrical resistance data were treated using principal component analysis (PCA). Fig. 8.4A shows a schematic illustration of the experimental setup of the e-tongue and the modified IDEs employed for analyzing milk samples. The e-tongue composed of sensors based on electrospun NFs distinguished contaminated solution from uncontaminated ones, even at very low concentrations for bovine milk with different fat contents. Moreover, the system was able to separate the samples by the antibiotics. The e-tongue showed high sensitivity, representing a less time-consuming alternative for other sophisticated analytical techniques.

The use of pesticides in agriculture is an increasing concern since they can be found in fresh foods that are ready to be consumed. An important class of pesticides employed worldwide is the organophosphate (OP) compounds. Such pesticides irreversibly inhibit the enzyme acetylcholinesterase (AChE) and therefore may cause serious health problems and even death [78]. A reliable approach to detect OP

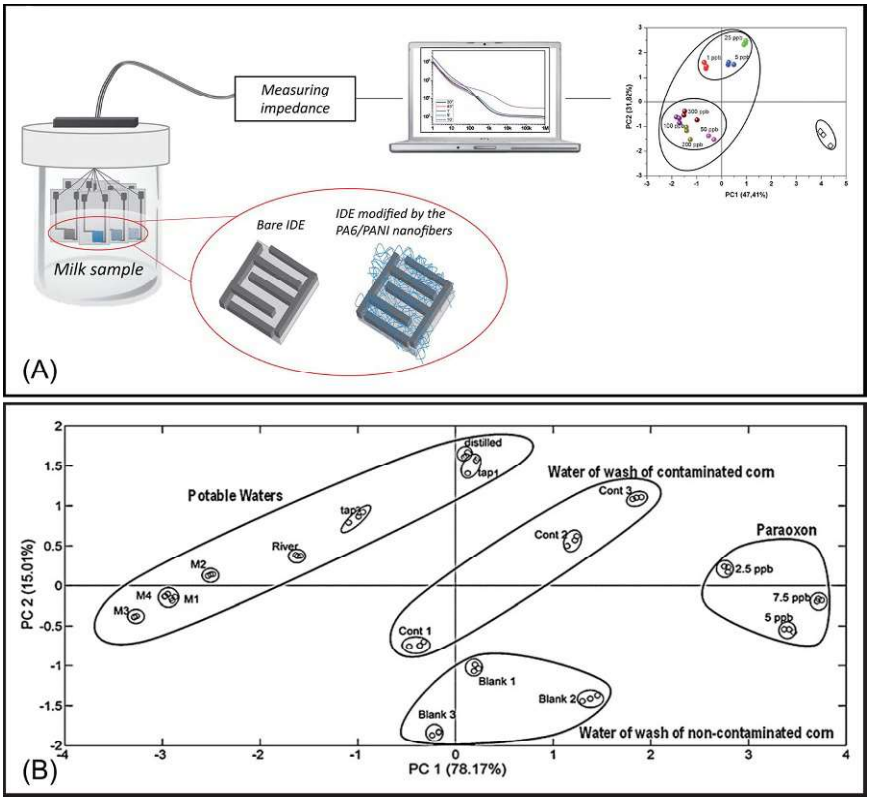

Fig. 8.4 (A) Experimental setup of the e-tongue used to analyze milk samples regarding the presence of antibiotic tetracycline and the PCA plot obtained with the experimental data collected. (B) PCA plot for 17 water samples analyzed by the electronic tongue and the discrimination in well-separated groups of samples obtained from different sources. Part (A): Adapted and reprinted with permission from V.P. Scagion, L.A. Mercante, K.Y. Sakamoto, J.E. Oliveira, F.J. Fonseca, L.H.C. Mattoso, et al., An electronic tongue based on conducting electrospun nanofibers for detecting tetracycline in milk samples, RSC Adv. 6 (105) (2016) 103740–103746. doi:https://doi.org/10.1039/C6RA21326J (web archive link). Copyright 2016 Royal Chemical Society. Part (B): Adapted and reprinted with permission from J.E. Oliveira, V.P. Scagion, V. Grassi, D.S. Correa, L.H.C. Mattoso, Modification of electrospun nylon nanofibers using layer-by-layer films for application in flow injection electronic tongue: detection of paraoxon pesticide in corn crop, Sens. Actuators B Chem. 171–172 (2012a) 249–255. doi:https://doi.org/10.1016/j.snb.2012.03.056 (web archive link). Copyright 2012 Elsevier.

pesticides is the development of electrochemical biosensors based on the inhibition of the AChE. El-Moghazy et al. made use of a nanofibrous membrane of chitosan (CS) and poly(vinyl alcohol) (PVA) to immobilize genetically modified acetylcholinesterase onto the surface of screen-printed electrodes in order to detect pirimiphos-methyl, an OP pesticide, in olive oil [79]. The use of NFs allowed enhancing the biosensor

response because enabled a higher immobilization of AChE molecules, facilitating the access of the substrate to the enzyme. Solutions of pirimiphos-methyl-oxon in concentrations ranging from 1×10^{-10} to 8×10^{-9} mol L^{-1} were prepared and the biosensor detection limited was found to be 0.2 nmol L^{-1}. Moreover, the biosensor showed excellent stability and reproducibility. The pesticides were then spiked into commercially available bio extra virgin olive oil and the analyses were made after a simple liquid-liquid extraction. All the recovery rates obtained for three different concentrations presented values very close to 100%, demonstrating the remarkable potential of the biosensor for food monitoring.

To detect hazardous OP pesticides in corn crop, Oliveira et al. used the layer-by-layer (LbL) technique to modify PA6 electrospun NFs with conductive polypyrrole (PPy) and poly(o-ethoxyaniline) (POEA) films to compose an e-tongue [80]. Such a system was assembled to a flow-analysis setup, which allows the processing of a large number of samples of small volumes in a short period of time. The sensing material was deposited onto graphite interdigitated polyethylene terephthalate (PET) substrates, producing seven sensing units by varying the number of PPy/POEA bilayers. By using the electrical resistance data in the PCA treatment, the system was able to identify the presence of the paraoxon pesticide in water samples obtained by washing the contaminated and noncontaminated corn. This sensor array represents an alternative approach to identify and analyze OP pesticides without making use of the AChE, avoiding some drawbacks, such as enzyme instability, extra costs, and tedious preparation. Other artificial prepared samples with a concentration of paraoxon pesticides as low as 2.5 ppb and water samples of varied sources, including potable waters, were also analyzed, being discriminated in well-separated groups on the PCA plot, as shown in Fig. 8.4B [80], showing the ability of the sensor to evaluate water quality and detect risky contaminants in food.

A fast and accurate way to determine the nutritional value of food, as well as the control of the presence of some important compounds for human health can assist the food industry in the preparation and acquisition of products with desired characteristics and compositions. Additionally, these analytical tools can also help the consumer in the choice of food [68]. Vitamin C is an essential vitamin to humans, a very important antioxidant that can prevent diseases and bring other great benefits to human health. Its presence in the human diet is indispensable and, therefore, precise methods for analyzing this type of compound in foods are required. Fuenmayor et al. developed a sensor for in situ selective determination of L-ascorbic acid (AA), the main biologically active form of vitamin C, in different types of foods [81]. The AA determination can be performed by some techniques, but in general, these methods are costly and time-consuming. In their work, a rapid, straightforward, and portable alternative that does not require major preparations is presented. A screen-printed electrode coated with nylon-6 nanofibrous membranes (SP-NFM) was used to estimate the content of AA of fruits directly in the sample. Foremost, the influence of the nanofibrous membrane in coating the electrode was evaluated through amperometric responses. Although it represented a decrease in sensitivity, due to the insulating nature of the membrane, the coating resulted in a widening of the linearity range. Since vitamin C can be found at different amounts in fruits, a wider linearity range extends the

applicability of the sensor. Furthermore, the coating reduced the effect of polyphenols present in the fruits under study, preventing distortions caused by this sort of compound in the amperometric responses. Different fruits, viz., apple, pears, lemon, kiwi, oranges, tangerines, and strawberries, with AA concentration between 30 and 600 mg kg^{-1} of the sample were analyzed by directly pricking the fruits with the SP-NMF electrode, proving that the sensor developed was suitable for in situ analyses of AA in many fruits.

Consumption of spoiled meat can be very harmful to the human health. In many cases, the determination of the quality of this type of food through sensorial analyses by the consumer, i.e., the evaluation of odor and appearance, maybe inaccurate or erroneous, leading to the ingestion of food not recommended for consumption and causing serious health problems [82]. When fish dies, for example, xanthine (Xa), a major metabolite in the degradation of adenosine triphosphate, starts to accumulate continuously in the fish meat. Therefore, the evaluation of the freshness of fish meat can be determined by the concentration of Xa. Tang et al. proposed a nonenzymatic Xa electrochemical sensor to evaluate the freshness of fish meat by performing measurements through real biological samples [83]. When biosensors make use of the xanthine oxidase enzyme, a specific determination can be hampered, since it also responds to hypoxanthine. To overcome this obstacle, carbon nanofibers (CNFs) with unique electronic properties were prepared by electrospinning polyacrylonitrile (PAN) solution and performing a subsequent thermal treatment. The CNFs were used to modify carbon paste electrodes (CPEs) and through electrochemical studies, the selective determination of Xa was achieved. When compared to other enzymatic biosensors, the proposed system achieved a significantly lower detection limit (20 nmol L^{-1}), also lower than other nonenzymatic electrodes, besides a wide linear range. The choice for the nanofiber structure was decisive in the achievement of the obtained results because it provided a nanoscaled platform with a high-specific surface area, leading to a large number of active sites. To perform the fish freshness analyses, a fish meat extract was prepared. Samples in different conditions of storage were analyzed and it was observed that the concentration of Xa increased continuously with storage time. The experimental results showed that the proposed sensing system could be used for food quality control.

The use of an enzymatic method to detect a target analyte offers some advantages specially due to the specificity, and sensitivity reached. However, in order to achieve enhanced performance, it is important to ensure that the immobilization will render electroactive and stable enzyme sites in a high population density. Marx et al. used electrospun gold nanofibers to immobilize fructose dehydrogenase (FDH) to detect fructose [84]. Gold nanofibers were chosen because their high surface area can serve as a useful platform for enzyme stabilization, aiming at enhancing the sensitivity of the sensor. To obtain the nanofibers, a solution containing PAN and chloroauric acid ($HAuCl_4$) was electrospun. The resulting fibers were then chemically reduced by immersion in sodium borohydride ($NaBH_4$) solution to create small metallic gold regions on the fiber that served as nucleation seeds for the electroless gold deposition process that followed. When compared to a flat gold disc surface, the immobilization of FDH onto gold nanofibers resulted in more active enzymes due to the

microstructure of the gold fibers. The FDH-gold fiber electrode exhibited a proportional response to fructose concentrations in the range of 0.1–3 mmol L^{-1}, with a limit of detection (LOD) of 11.7 μmol L^{-1}. To demonstrate the practical application of the sensor, the nanofibrous fructose sensor was tested in popular beverages. The responses of the sensor were compared with the results obtained using a commercial fructose kit. The correlation between the values demonstrated the precision and accuracy of the sensor. Furthermore, the sensor was also used to measure glucose by using the enzyme glucose isomerase, which catalyzes the isomerization of glucose to fructose, demonstrating the versatility of the sensor developed.

8.3.2 Sensors for detecting microbial contamination in food samples

Among sources of biological contamination, those caused by pathogenic microorganisms can be highlighted as a constant threat that can occur at all stages of the food chain, since pathogenic microbes can potentially be found anywhere and introduced directly or indirectly into food [66, 85]. Foods, in general, are a suitable environment for the growth of different types of microorganisms. Although some microorganisms can play a beneficial role in the food industry, pathogenic bacteria and viruses are the most significant for food contamination, which can cause several types of infections, leading to diarrhea, vomiting, etc. The most common types of foodborne pathogenic bacteria responsible for outbreak infections are *Salmonella* spp., *Staphylococcus aureus*, *Vibro cholera*, *Clostridium botulinum*, *Escherichia coli*, *Pseudomonas* spp. [85]. The undesirable presence of these microorganisms in food is a constant concern for the food industry and a real threat to human health. These facts highlight the importance of good practices for food production and preservation. In addition, the performance of chemical analysis during processing or before consumption of food to monitor and ensure quality and safety for consumers appears as an interesting option to provide safe food.

In this context, great efforts have been made to develop analytical techniques capable of detecting and identifying food contaminants, especially in trace amounts [86]. A variety of analytical techniques can be used to monitor food quality, but in general, they can be grouped into (i) spectroscopic, such as mass spectroscopy, nuclear magnetic resonance [87], infrared; (ii) biological as a polymerase chain reaction (PCR), immunological techniques [88] and biosensors; (iii) chromatographic such as high-performance chromatography, gas chromatography, capillary electrophoresis, and supercritical fluid chromatography; and (iv) electrochemical biosensors. Such techniques can be also associated (coupled) for best results [89, 90]. Regarding to foodborne pathogenic microorganism detection, the standard methods used in the food industry are PCR, cell culture and colony-forming units (CFU) counting, and immunoassays (ELISA). However, efforts in the development of molecular-based technologies for rapid, cost-effective, high-throughput, ease-of-use, and reliability in detection have emerged and research on the development of nanostructured biosensors for the detection of pathogenic microorganisms in food is increasingly being found [91].

Biosensors are a subgroup of chemical sensors in which a biological component (enzyme, antibody, nucleic acids, aptamers, etc.) is used for recognition of the target analyte. Such devices bring interesting advantages compared to traditional methods that combine fundamental concepts, engineering, and computer science to accurately meet the urgent demands of the food industry. Recently, researchers have demonstrated great interest in the use of composite nanomaterials to develop such devices in order to improve their performance and characteristics for food analysis applications [92]. Different types of biosensors have been reported in the literature for pathogen detection. Among them, the electrochemical-based biosensors are the most promising, which can be classified as a label-dependent and label-free, according to the detection technique used [93]. Ligaj et al. reported two electrochemical-based biosensors for the detection of the bacteria *Aeromonas hydrophila*. The first sensor was designed by the immobilization of DNA as a sensing element, which recognizes a specific gene associated with *A. hydrophila* presence, onto a gold electrode, using the self-assembled monolayer (SAM) technique. The second sensor was prepared using multiwalled carbon nanotubes (MWCNTs) for promoting covalent bonds between the DNA and the CPE. Both biosensors were successfully used for bacteria detection in fishes and vegetable samples and the results were correlated with those obtained with PCR [94]. Altintas et al. designed a fully automated microfluidic-based electrochemical biosensor for real-time *E. coli* detection in water samples. The sensor can be classified as a "sandwich immunoassay" developed by immobilization of polyclonal anti—*E. coli* antibody onto a gold surface electrode using SAM technique and a secondary antibody called horseradish peroxides (HRP) (referred to as a detection antibody which binds the *E. coli* bacteria in the epitope), in such a way that the bacterium remains "sandwiched" between the two antibodies. Tetramethylbenzidine (TMB) was used as a substrate once it can be oxidized in the presence of HRP. Gold nanoparticles (AuNPs) were used as signal amplifiers and a detection limit of 5 CFU mL^{-1} was achieved in water samples [95]. Wilson et al. developed a sensitive, rapid, and low-cost biosensor based in electrical impedance spectroscopy measurements to detect Gram-positive and Gram-negative bacteria such as *E. coli*, *S. aureus*, and *Salmonella typhimurium* in potable water and apple juice. Carboxylated Fe_3O_4 magnetic nanoparticles were synthesized and bioconjugated with the antimicrobial peptide melittin (MLT), which can recognize pathogenic species, and then coupled onto screen-printed interdigitated electrodes. The results showed that the biosensor was capable to detect *E. coli* within 25 min down to a few CFU mL^{-1} and also discriminated against the three types of bacteria presented in water and apple juice [96]. A label-free electrochemical impedance immunosensor for detecting *S. typhimurium* in milk samples was developed by Dong et al. A sensitive and stable biosensor was achieved by immobilization of anti-*Salmonella* antibodies in gold nanoparticles and poly(amidoamine)-multiwalled carbon nanotubes-chitosan nanocomposite film onto glassy carbon electrode. The biosensor exhibited a detection limit of 5.0×10^2 CFU mL^{-1} for *S. typhimurium* in milk samples and recoveries from 94.5% to 106.6% [97].

Among the (bio)sensors employed for the detection of pathogenic microbes in food, those based on electrospun NFs present enormous potential [39]. For instance,

electrospun NFs can contribute to the improvement of biosensing by increasing the surface area/volume ratio where the active materials are deposited or contained, leading to increasing contact between the sensing platform and the target analyte. Additionally, electrospun NFs are often employed as a composite material or even chemically surface functionalized, which brings not only advantages of the single nanomaterial but also combining desirable properties of the hybrid nanomaterials [39, 98].

Recently, the number of papers reporting the use of electrospun NFs as biosensors component for pathogenic microorganism detection has considerably increased. For instance, Table 8.2 summarizes some examples of electrospun NFs-based biosensors for foodborne pathogenic detection. This overview of recent reports comparing nanofibers composition, detection method, food matrix (when provided), and the LOD is presented to reinforce the potential of electrospun NFs for foodborne pathogenic detection.

Specifically, the applications of NFs in biosensing include lab-on-a-chip devices combining lateral flow assay and microfluidic technology [101, 108], paper-based point-of-care (POC) devices [102], membranes (mats) to specifically capture and concentrate the analytes for enhanced purification and detection [109], and biosensors associated with magnetic nanoparticles and/or with antibodies.

A colorimetric paper-based lateral flow assay (LFAs) is designed using functionalized electrospun NFs for *E. coli* O157:H7 cell detection [102]. The NFs are formed by poly(lactic acid) (PLA) and poly(ethylene glycol) (PEG) and used as matrices for anti-*E. coli* immobilization. *E. coli* biosensor platform was produced using a paper-based design and sandwich binding assay, as displayed in Fig. 8.5A, which shows the paper-based device formation where the NFs mat is placed directly on a backing card and an absorbent pad is placed on the backing card overlapping the NFs mat. Then, a primary *E. coli* antibody was immobilized onto NFs (red antibodies) and a secondary antibody (yellow antibodies) was conjugated with HRP enzyme [Fig. 8.5A(ii) and (iii)]. Fig. 8.5A(ii) shows that once *E. coli* cells (green structures) bind both antibodies, it is "sandwiched" leading to a colorimetric signal upon addition of the HRP substrate, detecting the bacteria presence. On the other hand, Fig. 8.5A (iii) shows that no signal is generated in bacteria absence. The color intensity results quantified using Image J software analysis are graphically displayed in Fig. 8.5B, whereby a detection limit of 1.9×10^4 cells was determined [102].

Xu et al. designed a label-free electrochemiluminescent (ECL) immunoassay based on magnetic functionalized electrospun NFs for the detection of aflatoxin B1 (AFB1). Once AFB1 is a toxin produced by *Aspergillus flavus* and *A. parasiticus*, the designed sensor is an indirect form to detect such fungus species. The ECL immunosensor was formed by Fe_3O_4 NFs magnetically immobilized on carbon nanohorns (CNHs) onto a magnetic glassy carbon electrode (MGCE). Aiming to specific analyte recognition, AFB1 antibody was immobilized on the modified electrode surface. The authors highlight that the use of NFs as a sensitive layer (on the interface) provides large numbers of binding sites to interact with the antibody, leading to high sensitivity and low LOD. The proposed immunosensor exhibited a detection limit of 0.02 ng mL^{-1} and was successfully used to detect AFB1 in corn samples [106].

Table 8.2 Electrospun NFs-based biosensors for detection of common microbial pathogenic of food.

Microbial target analyte	Nanofibers-based electrodes	Detection method	Food matrix	LOD	Ref.
Escherichia coli	[a]PCDA/[b]PEO/[c]PU NFs	Colorimetric	–	–	[99]
Escherichia coli K12	[d]PAA/[e]PVA NFs	Potenctiometry	Water	20 CFU mL^{-1}	[100]
Escherichia coli O157	[f]PVP NFs/[g]HRP	Chemiluminescence	–	10^6 CFU mL^{-1}	[101]
Escherichia coli O157:H7	[h]PLA-[i]PEG NFs/Anti-E. ColiO15:H7	Colorimetric	–	1.9×10^4 cells	[102]
Escherichia coli O157:H7 *BVDV (Bovine viral diarrhea vírus)*	[j]PVDC/[k]NC NFs/Anti-E.ColiO15:H7	Conductometric	–	61 CFU mL^{-1} 10^3 CFU mL^{-1}	[103]
Escherichia coli *Staphylococcus aureus*	[l]PAN/[m]pVDB NFs	Fluorescence	–	– –	[104]
Escherichia coli *Salmonella*	[n]ITO/[o]PCL NFs/Anti-*E. coli*	Fluorescence	–	10^2 CFU mL^{-1} 10^2 CFU mL^{-1}	[105]
Aflatoxin B1 (from *Aspergillus flavus* and *A. parasiticus*)	[p]MGCE/Fe_3O_4 NFs/[q]CNHs	Electrochemiluminescence	Corn	0.02 ng mL^{-1}	[106]
Aflatoxin B1 (from *Aspergillus flavus* and *A. parasiticus*)	[r]GCE/PAN-[s]LRC	[t]DPV	Poultry feed	–	[107]

[a] PCDA: 10,12-pentacosadiynoic.
[b] PEO: polyethylene oxide.
[c] PU: polyurethane.
[d] PAA: poly(acrylic acid).
[e] PVA: poly(vinyl alcohol).
[f] PVP: polyvinylpyrrolidone.
[g] HRP: horseradish peroxides.
[h] PLA: polylactic acid.
[i] PEG: polyethylene glycol.
[j] PVDC: polyvinylidene chloride.
[k] NC: nitrocelulose.
[l] PAN: polyacrylonitrile.
[m] pVDB: poly(4-vinylphenylboronic acid-co-2-(dimethylamino)ethyl methacrylate-co-*n*-butyl methacrylate) magnetic nanoparticles.
[n] ITO: indium doped tin oxide.
[o] PCL: polycaprolactone.
[p] MGCE: magnetic glass carbon electrode.
[q] CNHs: carbono nanohorns.
[r] GCE: glassy carbono electrode.
[s] LRC: Lake Red C
[t] DPV: differential pulse voltammetry.

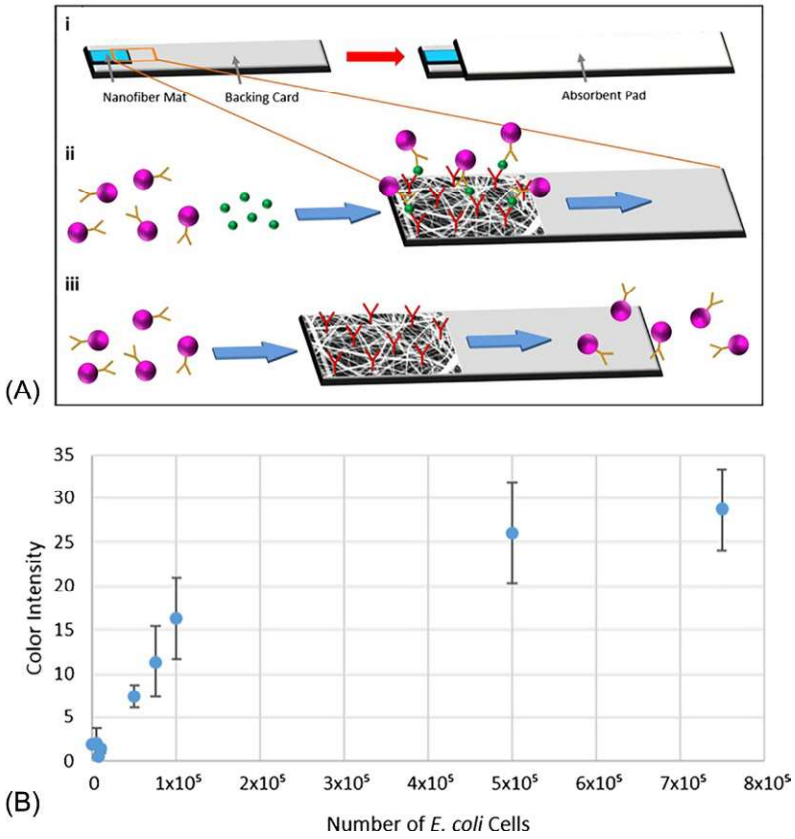

Fig. 8.5 (A) Schematic representation of LFA format and sandwich-binding assay. A 1.75 × 5 mm nanofiber mat is placed directly on a backing card 4.5 mm in width, and a 1 × 20 cm absorbent pad is placed on the backing card overlapping the nanofiber mat by approximately 2 mm (i). The LFAs run vertically in glass culture tubes. In the *E. coli* sandwich assay, *E. coli* (green—gray color in print version) flow through the anti-*E. coli* modified nanofiber mat, followed by horseradish peroxidase (HRP)-conjugated (pink—dark gray color in print version) anti-*E. coli*. When *E. coli* is present, a colorimetric signal results upon the addition of HRP substrate (ii), and when no *E. coli* is present, the HRP antibodies flow through the nanofiber mat and no signal is observed (iii). (B) Nanofiber-enhanced *E. coli* LFA signal dose-response curve. Anti-*E. coli* antibodies are immobilized on PLA-PEG nanofibers. A sandwich assay is performed in which *E. coli* bounds to the capture antibody, and a reporter antibody conjugated with HRP bounds to the captured *E. coli* cells to produce a colorimetric signal when substrate is added.

Adapted and reprinted with permission from S.J. Reinholt, A. Sonnenfeldt, A. Naik, M.W. Frey, A.J. Baeumner, Developing new materials for paper-based diagnostics using electrospun nanofibers, Anal. Bioanal. Chem. 406 (14) (2014) 3297–3304. https://doi.org/10.1007/s00216-013-7372-5 (web archive link). Copyright 2014 Springer.

A simple, rapid, cost-effective, and high sensitivity sensor for *E. coli* detection was developed using a pH-sensitive hydrogel NFs integrated with a light addressable potentiometric technique (NF-LAPS) [100]. Electrospun NFs formed by polyacrylic acid/polyvinyl alcohol (PAA/PVA) were used as sensing layers for changes in pH environment (acidity increases) caused by *E. coli* sugar fermentation. A high sensitivity sensor toward *E. coli* was achieved exhibiting a LOD of 20 CFU mL^{-1}, which was attributed to the NFs pH-sensitive behavior. The advantages of the system rely on the nonrequired biological component (enzyme/antibody-antigen) leading to a cheaper, less time consuming, and more practical device. Moreover, the selectivity toward *E. coli* detection was ensured using D-mannose, which was attached on NFs surface using divinyl sulfone as a cross-linker, leading to a selective binding.

Jin et al. designed a capillary flow microfluidic with on-chip reagent delivery which combines a lateral flow assay with microfluidic technology for *E. coli*. detection [101]. Such a device employs polyvinylpyrrolidone (PVP) NFs as water-soluble storage for HRP enzyme. HRP tagged antibody was electrospun with PVP and stored in a microfluidic poly(methyl methacrylate) (PMMA) chip. Fig. 8.6A shows the picture of capillary flow microfluidic with nanofibers placed in the chamber. Briefly, when the *E. coli O157* sample makes contact with NFs, it releases reagents for binding. The dose response for the microfluidic chip is quantified using chemiluminescence, as displayed in Fig. 8.6B, and the LOD is found to be approximately 10^6 CFU mL^{-1} of *E. coli* O157 [101].

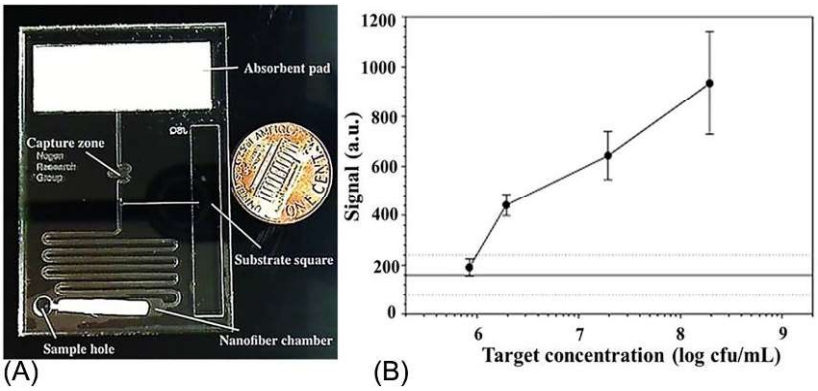

Fig. 8.6 (A) The picture of capillary flow microfluidic using nanofibers. The sample treated with magnetic beads is added to the sample hole and reacted with antibody inside nanofibers, the solution go through the channels by capillary, and the magnetic beads are isolated in the capture zone. The absorbent pad generated a continuous flow, made the washes, and substrate travel through channels. (B) *Escherichia coli* O157 detection in capillary flow microfluidic. The solid line is the blank detection, while the dotted line is the blank added standard deviation.
Adapted and reprinted with permission from S. Jin, M. Dai, B. Ye, S.R. Nugen, Development of a capillary flow microfluidic *Escherichia coli* biosensor with on-chip reagent delivery using water-soluble nanofibers, Microsyst. Technol. 19 (12) (2013) 2011–2015. https://doi.org/10.1007/s00542-013-1742-y (web archive link). Copyright (2013) Springer.

8.4 Summary and perspectives

Food contamination is of utmost concern and imposes a series of challenges in terms of food safety owing to the huge demand for food production and food supply for the growing population. Besides, regulations are not always fulfilled during food production and storage, which make the scenario even more critical. In this way, food sensors employing composite nanofibers as active layers, operating under varied transduction mechanisms, are a key element to provide remarkable improvements in terms of sensor sensitivity and LOD. The functionalization of nanofibers with other active materials opens a new opportunity for food sensors capable to detect varied contaminants such as pathogenic bacteria and pesticides in an expedited way. Additionally, such nanofibers can be integrated into miniaturized and low-cost flexible sensors for in situ analysis. However, the fabrication of such composite nanofibers at large scale with well-controlled properties for sensors application imposes some challenges to be overcome in order to allow its widespread use in industrial and commercial applications. In this direction, although the SB-spinning technique also requires further technological advances, its high nanofiber output rate is potential for scaling-up the fabrication of nanofibers-based sensors for food analysis.

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