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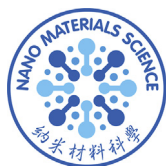
Advances on ultra-sensitive electrospun nanostructured electrochemical and colorimetric sensors for diabetes mellitus detection

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ABSTRACT

Recent developments in the biochemical and medicinal industries have been heavily focused on producing affordable glucose biosensors due to the continuous annual increase of diabetic patients worldwide. The development of a fast, accurate, and reliable glucose sensor will increase confidence in controlling diabetes mellitus and its associated health complications among the diabetic community. Electrospinning is a versatile method that can produce complex nanofibrous assemblies with attractive and functional characteristics from various polymers. Electrospun nanofibers demonstrated high efficiency in the immobilization of biological molecules, which can improve the sensing performance further. Integration of polymer electrospun nanofibers with metal nanoparticles, metal oxide or transition metal in producing nanobiocomposites is also a highly popular approach in the past few years. This report presents the current progress and research trends of the technique, focusing on various materials and fabrication strategies used to produce biosensing interfaces. This helps readers decide the suitable approach in designing highly sensitive, selective, fast, and inexpensive glucose sensors.

1. Introduction

Diabetes mellitus is a chronic disease and metabolic disorder, which causes blood glucose levels to increase due to defects in insulin secretion, insulin action, or both [1]. Patients are considered diabetic if two consecutive fasted blood glucose tests indicate a blood sugar level of 126 mL/dL or higher. Normal blood sugar levels are less than 100 mg/dL [2]. On a global basis, the number of people with diabetes is around 450 million and is expected to increase by an additional 50 million by the year 2035 [2]. There are two different phenomena of glucose levels in the human body. The first phenomenon is low glucose levels, known as hypoglycemia, where the blood sugar decreases below the normal level, which leads to fainting, coma, and even death [3]. The second phenomenon is abnormally high blood glucose levels, known as hyperglycemia, which negatively affects the eyes, kidneys, nerves, and blood vessels [4].

Due to the increasing number of diabetes patients globally, more attention has been given to develop highly sensitive and selective glucose biosensors for fast detection. Although several techniques have been proposed for glucose monitoring sensors, more attention is given to electrochemical sensors due to their simplicity, inexpensiveness, ability to integrate with portable devices, and to offer quantitative analysis. Furthermore, the electrochemical glucose sensing mechanism can be categorized as enzymatic glucose sensing and non-enzymatic glucose sensing. During enzymatic glucose sensing, glucose molecules are oxidized as gluconic acid using the GOx enzyme. Herein, the GOx enzyme is considered an ideal enzyme for glucose oxidation [5] due to its high sensitivity, precise selectivity, and high stability comparing with other enzymatic materials [6]. Ideally, to achieve high sensitivity and longevity in glucose biosensors, hydrogen peroxide, which is the product of glucose oxidation to gluconic acid, must be paid attention to, as it can deactivate GOx [6]. Generally, the fabrication of glucose sensors requires immobilization of enzymes and probe molecules on a suitable matrix, through physical adsorption, covalent attachment, or physical entrapment of encapsulation [7]. Enzyme immobilization is the attachment of an enzyme to inert and insoluble materials, which provide an increase in resistance towards the changes of pH or temperature, reusability, a continuous operational mode, and possible modulation of the catalytic properties compared to dissolved enzymes. The enzymes are held in place throughout the reaction and can later be easily separated from the

end products for reusability [8]. However, the complex immobilization procedures often lead to poor reproducibility and performance on the sensor matrix [8]. Unfortunately, maintaining the presence of the mediator near the electrode and the enzyme surface is very difficult over a long period of usage due to their relatively small size and diffuse molecules. The formation of the irritating hydrogen peroxide as an interference species was also reported [7].

A recent generation of enzymatic electrochemical sensors applies for direct electron transfer (DET) between their enzymes and the electrode, without the need for natural or synthetic mediators. Despite the advantages of such a high sensitivity, selectivity, and fast response of DET sensors, higher production cost, and thermal instability caused by the enzymes are significant challenges [9]. Furthermore, the tedious nature of enzyme immobilization methods and their critical operating conditions add to their disadvantages. Enzymatic activities are more likely to be influenced by external parameters (pH, temperature, humidity, and toxic chemicals). Non-enzymatic sensors are labeled as the fourth-generation sensors; however, due to their relatively low kinetics of glucose electro-oxidation on conventional electrodes, these sensors still pose some challenges in terms of glucose sensing sensitivity [10]. Furthermore, the irreversibly adsorbed oxidation intermediates of glucose and adsorbed chloride ions cause the impairment of noble metal electrodes [11]. Non-enzymatic glucose sensors are also considered relatively poor in selectivity [12], which could potentially oxidize some other sugars and interfering species in the potential range of glucose oxidation [10]. Moreover, these sensors are unstable and experience frequent fouling of electrodes, making them unfit for practical applications [13–15]. However, these drawbacks can be overcome by incorporating a suitable solid matrix with catalytic material of non-enzymatic sensor or applying nanocomposite-based catalytic materials for non-enzymatic glucose sensing applications. Significantly, electrospun nanofiber-based composite structures are considered as a promising candidate for improving sensitivity and selectivity of electrochemical sensors by offering large catalytic surface area, encapsulate a high amount of enzymes, and the ability to incorporate with a wide range of catalytic components.

Colorimetric sensors for glucose sensing are gaining increasing interest due to their simplicity and low cost. Besides, other glucose sensors including enzyme- and non-enzyme-based electrochemical glucose sensors are operated based on the calibration curve method, which involves complicated data-collection and processing features, and these sensors require specific and advance scientific equipment. Alternatively, the colorimetric method proves to be a promising method that overcomes the drawbacks of electrochemical sensors due to simplicity, better ability for high throughput analysis, and continuous monitoring. In addition, colorimetric sensors indeed have become a trend in rapid and semi-quantitative determinations. Its advantage lies in the change in color occurring due to the presence of analytes that can be read by the naked eye without any usage of complicated equipment or professional operators [16,17]. These features of colorimetric sensors demonstrate the ease of their usability for field analysis and point-of-care-diagnosis [18–20]. Colorimetric detection of analytes, including hazardous chemicals, toxic metal pollutants, and explosives, has been extensively investigated in the recent past [21–23]. Up to date, most of the colorimetric sensors are designed to disperse in target sample solutions for achieving maximum contact with target molecules [24]. However, the uneven dispersion of colorimetric sensors and unstable attachment of probe molecules and target molecules often affect the authenticity of results. Avoiding these drawbacks, a variety of solid matrices have been reported for the ability to enhance the performance and stability of colorimetric probes, leading to the improvement of the overall sensing performance for various field applications [25–27]. Also, the solid matrices serve as an ideal platform for the enzymes' retainment of their stability against the varying surrounding conditions and providing easy accessibility to probe analytes. However, most of the selected support persistently affects the reactivity and sensitivity of the sensing performance due to enzymatic properties.

Fortunately, with the development of electrospun nanofibers, it proves to dismiss all the disadvantages listed due to its large surface area that offer a large active area that improve sensitivity [28]. Furthermore, controllable structure, highly porous surface, and excellent flexibility of electrospun nanofiber enhance the probe immobilization rate and stability of the sensor [25,27].

The composition of nanomaterials in electrochemical and colorimetric sensors played an important role in determining their performance in a catalytic process. Various research groups have shown evidence that different architecture of nanomaterials does indeed enhance the performance of glucose detection and monitoring [29,30]. The stability of these nanomaterials is given great attention in the aspect of controlling their sizes and avoiding agglomeration by having a robust support system for the characteristic retainment of individual particles. A great focus has also been given to the reproducibility of nanomaterials' production [31–33]. Moreover, the capability of encapsulating many enzymes and catalytic agents while offering a large surface area for the chemical reaction significantly enhances the sensor's sensitivity and selectivity. The electrospinning procedure is reported to be relatively low-cost in the large-scale production of nanofibers. Electrospun nanofibers and their composite materials offer a large surface area to volume ratio, porous structure, high flexibility, and interconnectivity enzyme immobilization, leading to excellent output in the glucose-sensing area [6]. Besides, electrospun nanofibers are one of the popular, versatile materials that can be used for both *in vivo* and *in vitro* glucose monitoring applications. Significantly, the reproducibility, biocompatibility, high mechanical strength, and optimizable degradability properties of electrospun nanofibers confer electrospun composite nanofibers as a potential candidate for continuous glucose monitoring in *vivo* applications. These include surgical implantation and non-invasive wearable biosensors that quantify the glucose level present in the saliva, tear, interstitial fluid (ISF), sweat, and blood.

This review aims to give an update on the progress of electrochemical and colorimetric nanobiosensors fabricated by electrospinning in recent years. Studying the electrospinning process method for fabricating glucose sensors and examining the effects of different materials on the functions and performance of the sensors will help the reader better understand the challenges faced in this novel technique. Additionally, the qualitative and quantitative comparisons among the different fabricated sensors in the discussion and analysis section of this paper provide insight into the chemistry and biochemical, and chemical engineering communities to produce high-quality, reliable glucose biosensors under optimum conditions.

2. Biosensor fabrication by electrospinning

Electrospinning is an efficient, convenient, and powerful technique for fabricating the nanofibers with diameters in the range of 100 nm and 1 μm at a large scale from a rich variety of polymers [34]. The conventional electrospinning process involves using a nozzle with a polymer flow that is subsequently causing a fine, charged polymer of a jet to be ejected from the tip of the conical shape electrified polymer interface namely, Taylor cone (Fig. 1) [35]. This jet extruded towards the counter electrode is bending due to the instabilities in the electric field, leading to a spiral movement in space [36]. The charged fiber is then accelerated towards the counter electrodes, which then thins rapidly due to the elongation and evaporation of the solvent until solid fibers are deposited onto the substrate on the top of the counter electrode [37,38].

Electrospinning processes can be easily modified to control fiber diameter, morphology, and porosity by manipulating the electric or magnetic field, alternating current or air-based fiber, incorporation of sacrificial porogen/sacrificial fiber, and cryo-electrospinning [39]. The size and morphology of the electrospun and electrospray products can be controlled by the following governing factors: the electrospinning setup parameters, polymer solution properties, and ambient conditions. The electrospinning setup parameters consist of the electrical potential, the
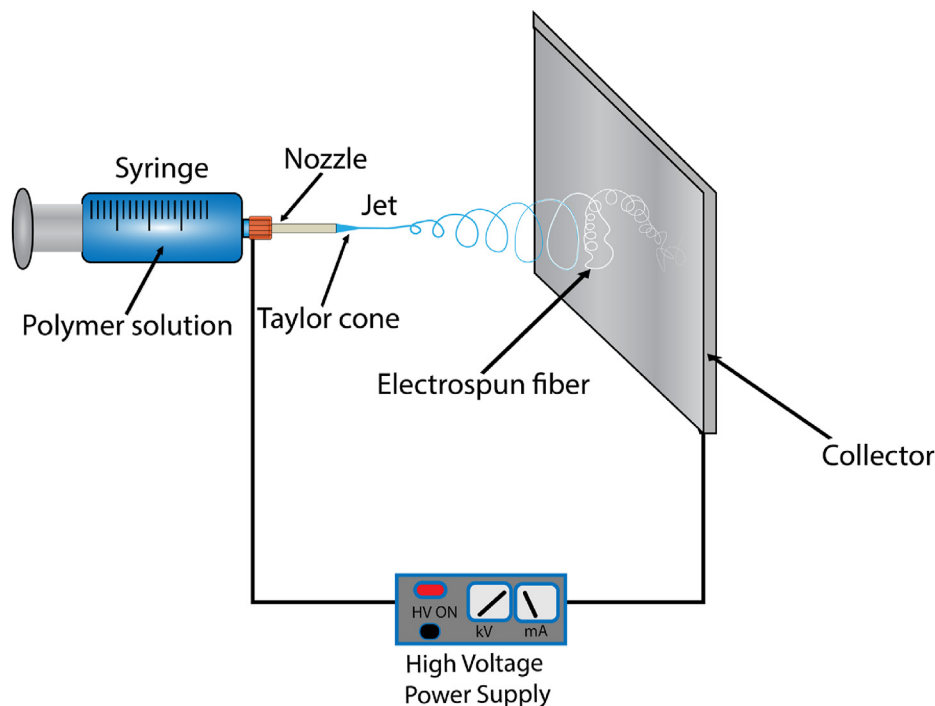
Fig. 1. Schematic illustration of the electrospinning process.

solution flow rate, the nozzle diameter, the collector geometry, and the working distance (distance between the nozzle and collector. Solution properties such as molecular weight, pH, solution conductivity, viscosity, and surface tension are determined by the type and concentration of the polymer and solvent used. Additionally, the ambient conditions such as the temperature, humidity, and air velocity in the electrospinning chamber determine the rate of evaporation of the solvent from the electrospun product [40–42]. Fibers that are formed from low viscosity solutions do not have a uniform diameter along their length, and often contain beads [43]. By varying the polymer concentration, feeding rate, and applied voltage, fibers with homogeneous diameters of below 1 μm can be fabricated. It is even possible to achieve fiber diameters of a few nanometers with an enhanced electrospinning setup [44].

Bead-free and uniform electrospun nanofibers are desired because

they display high surface area, porosity, flexibility, high mechanical and structural properties, low basis weight, and their production is cost-effective [45]. The high surface area allows electrospun nanofibers to accommodate large immobilization sites, leading to increased interaction with analytes. A combination of different functional particles into a nanofiber matrix can be achieved through the electrospinning process. These functional particles will either disperse into the polymer solution and then be electrospun to produce composites in the form of continuous nanofibers (in-situ) or coat on the nanofibrous assemblies by an additional process (ex-situ). Both products contain enhanced manufacturing capabilities and carry great flexibility, which eases the handling and maintenance of their reproducibility [46]. Substrates with high surface area, porosity, and chemical inertness properties are essential to obtain high sensitivity and enhanced performance for sensing and precise
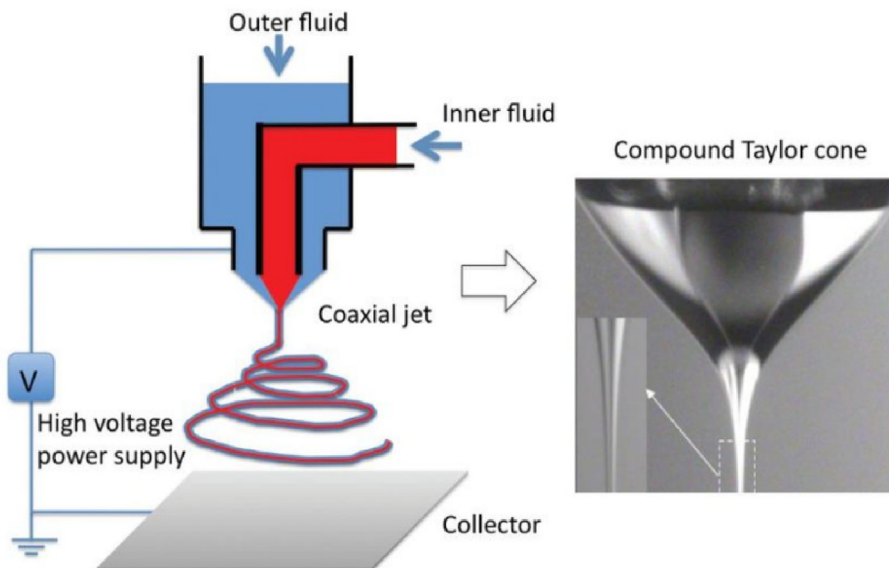

Fig. 2. Basic configuration for coaxial electrospinning core-shell nanofibers [49].

measurement [45]. The nanoparticles usually limit the mass transfer rate of analytes. However, electrospun fibrous membranes are proven to be highly efficient in immobilizing biological molecules with higher loading capacity, higher stability, and a longer lifetime of enzymes, further improving their sensing performance [47,48].

Coaxial electrospinning, as depicted in Fig. 2, involves two concentrically arranged nozzles connected to a power source. Two different solutions with different polymers and solvents can create products with tailor-made properties if the process is controlled properly [49]. Such core-shell structured nanofibers can be achieved using coaxial electrospinning, producing complex multi-level structures from a single component [50]. This technique has many applications in biomedicine [51], optoelectronic devices [52], absorption filtration [53], and tissue engineering [54]. Coaxial electrospinning has been used previously for coating a miniature coil-type implantable glucose sensor [55]. In this study, electrospinning was performed directly on the sensor surface to produce uniform fibro-porous coatings around miniature ellipsoid biosensors (Fig. 3). Apart from that, coaxial nanofibers provide a well-controlled material environment that reduces the leaching of enzymes that is entrapped inside the core area of nanofiber in enzyme-based glucose sensors [56]. This efficient enzyme encapsulation strategy improves the durability and functionality of glucose sensors. Furthermore, coaxial electrospinning allows the encapsulation of multiple different types of reactants such as enzymes or nanoparticles in a single nanofiber. These nanofibers release the reactants during the glucose monitoring process whereby these reactants will react with each other or target molecules to produce sensing signals such as color change or free electrons. Despite the advantage of coaxial electrospinning, the main drawback of this approach is, a high enough chain-entangling density in the working solution is necessary to prevent capillary breakup and Rayleigh instability, leading to the production of lower quality nanofibers [57]. A common approach for the preparation of coaxial composite nanofibers with superior morphology is to reduce the electrospinning solution to the lowest possible concentration [50,57]. Other approaches to producing thinner nanofibers include manipulating intrinsic spinning solution properties, adding additives (salts and surfactants) to the polymer solutions, and controlling the electrospinning process parameters [58].

3. Fabrication processes and materials used for synthesizing electrospun electrochemical biosensors

Conducting polymer-based composite material and nanoparticle-based composite materials are generally used to fabricate electrochemical biosensors due to their conductivity, low resistance, biocompatibility and, high mechanical and chemical strengths. Various procedures such as soft/photolithography, chemical, and enzymatic methods have been proposed to fabricate a conducting polymer-based sensor matrix [59–61]. Generally, lithographic methods require high energy, and this high-energy environment does not allow the doping of biomolecules with conductive polymers. On the other hand, chemical methods demand high monomers concentrations and a strictly controlled pH environment [62]. In addition, chemical synthesis usually uses toxic catalysts or strong oxidizing agents [63]. As a substitute for conventional lithographic and chemical methods, more attention is given to the electrospinning method for synthesizing polymer-based composite fibers for sensor applications due to their simplicity, desirable features, biocompatibility, and cost-effectiveness. Generally, electrospun composite nanofibers offer nanometric diameter and high porosity that confers large surface area to sensor matrix [64]. Also, the 2D structure of electrospun polymer composite nanofibers decreases the grain energy of the deposition and increases the electron diffusion coefficient that efficiently increases the signal amplification of the sensor [65]. Furthermore, unlike traditional methods, the electrospinning method is a low-energy wet synthesis method that simplifies the layer by layer assembly or doping of nanoparticles during the sensor matrix preparation [34].

Besides, the usage of nanoparticles was proven to enhance the performance of the biosensors analytically. The large surface area of the nanoparticles allows more convenient access for the specified analytes, further enhancing the sensing performance. The combination of nanoparticles is deemed possible due to the structural size of the particles, allowing the implementation of desired features into the biosensors [66]. Besides, nanoparticle-based polymer composites, in which polymers are mixed with nanoparticles, display advantageous properties in the electrical, optical, mechanical, and thermal aspects [67,68]. Nanoparticle-based polymer composite samples show enhanced stability, flexibility, and anatomy ability to retain the individual nanoparticle traits in the formation of complex structures [69]. The polymer network acts as
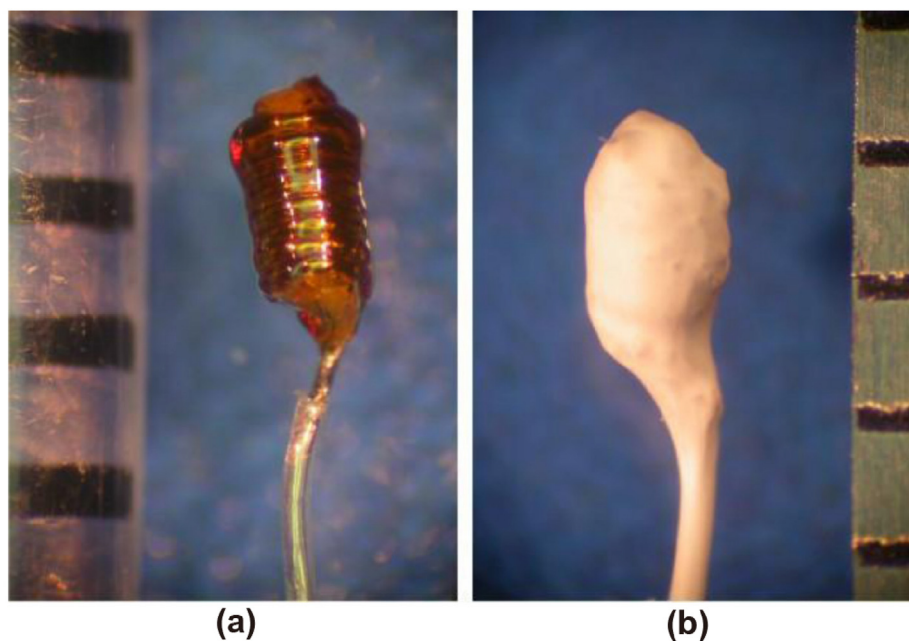

(a) **(b)**

Fig. 3. Optical microscope images showing the morphology of a coil-type implantable glucose biosensor without (a) and with (b) the electrospun coating. Scale bar is 1 mm [55].

a template medium that stabilizes the nanoparticles on a long-term basis, protecting their functionality. The sensing performance of the biosensor largely depends on the compatibility and interaction between the nanoparticles and polymers [70]. To obtain the expected function of the composites, the homogeneous distribution of nanoparticles in the polymer matrix has to be ensured, as nanoparticles tend to aggregate and lose their functionality [71]. Hence, electrospinning seems to be a promising and effective method to synthesize the nanoparticle-polymer composite sensor matrix for sensor applications. Self-repelled discrete, nanometric diameter, and highly porous features of electrospun nanofiber allow nanoparticles to be well distributed and dispersed inside the electrospun fibers. Methods of synthesizing various types of composite sensor matrix by electrospinning are briefly described in Fig. 4.

3.1. Polymer and carbon nanofibers for glucose detection

In recent days, several electrospun glucose-sensing platforms have been proposed by using the advantage of mixtures of polymers such as poly (vinyl alcohol) (PVA) [72], poly (vinyl pyrrolidine) (PVP) [73], polyethyleneimine (PEI) [74], polyacrylonitrile (PAN) [75], and cellulose [76] materials. These materials are capable of retaining the activity of the enzymes, leading to the usage for sensitive electrochemical detection of glucose. Generally, PVA, PVP, and PEI are hydrophilic, non-toxic, and highly biocompatible synthetic polymers, easy to electrospun, and provides a favorable microenvironment for the enzymes [77–79]. Besides, carbon-based nanofibers synthesized by the electrospinning method gain considerable attention for glucose sensing applications. Generally, carbon-based electrospun nanofibers are produced via two methods. 1. Electrospinning the polymer solution followed by high-temperature pyrolysis of electrospun polymer fibers, 2. Directly electrospun the carbon-based nanomaterial such as graphene [80], carbon nanofibers (CNFs) [81], carbon nanotubes (CNTs) [82], and carbon foams [83] mixed into polymer precursor solutions. These carbon-based electrospun nanofibers possess high surface area [84], providing a larger amount of electron transfer sites [85], surface functionalization [86], enzyme loading [87], and providing a platform for additives [88]. In addition, carbon nanomaterials offer high thermal [88], mechanical [89], and chemical [90] stability which confer high stability for enzymatic and non-enzymatic sensors. Glucose sensors with enhanced performance are developed by immobilizing biomolecules onto the surface of the electrospun carbon structures.

PEI, a water-soluble polymer, is a suitable matrix for enzyme stabilization due to its abundance in protonated primary amino groups, which can interact with anionic groups at the surface of proteins [91].
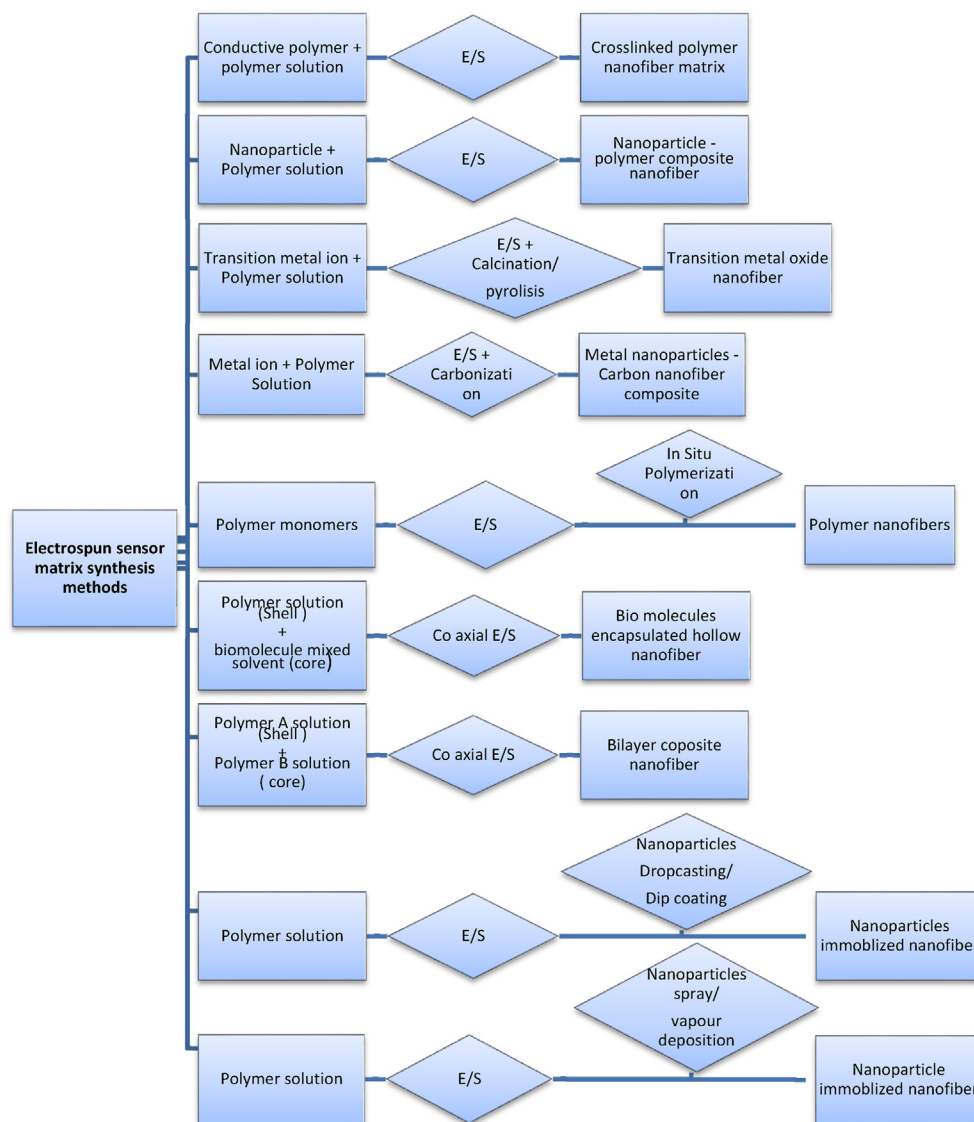

Fig. 4. Different synthesis methods of composite electrospun nanofibers for electrochemical and colorimetric sensor matrix.

Unfortunately, the presence of both charged primary amino groups and secondary amino groups simultaneously in the PEI chain lead to the formation of inter and intramolecular hydrogen bonds, which negatively affects the electrospinning process of PEI solution [92]. It is then proposed that a mixture of PEI and PVA can weaken the hydrogen bonding in the PEI system by favoring the interactions between imine and amine groups of the PEI chain and hydroxyl groups of the PVA chain [92]. The proposal leads to a great increase in conductivity, viscosity, and surface tension of the PEI solution, enhancing their electrospinnability and thermal stability of PVA/PEI mixture compared to PVA alone [64,66]]. PEI in the PEI/PVA mixture brings reactive amino groups, helping in anchoring the produced nanofibers to various surfaces and cross-linking the fibers to render them insoluble in aqueous media [93]. However, to date, only one research group managed to develop PVA/PEI/enzyme nanofibers (NFs) electrochemical biosensors by employing horseradish peroxidase, adsorbed onto the fabricated PVA/PEI NFs, for glutathione, hydrogen peroxide, or glucose detection [94,95]. An ultimate drawback reported from this group of researchers is that only the external surface of the NFs is used for enzyme immobilization, showing low usage efficiency, as the internal volume of the fibers is not involved in the immobilization. This approach allows enzyme loading to be limited and easy desorption of the biomolecules from the surface upon repetitive sensor usage [94, 95].

Polyvinyl alcohol (PVA) is a polycrystalline material widely used in hydrogel-based biosensor application due to its biocompatibility, high chemical stability, and hydrophilic characteristics [96]. Recently, Kim et al. fabricated a glucose biosensor by blending and electrospinning PVA and PAA solutions [97]. The PVA/PAA nanofibers were heated to 160 °C to cross-link the –OH groups in PVA and the –COOH groups in PAA. Glucose oxidase (GOD) enzyme was immobilized by the physical absorption of the enzyme onto the PVA/PAA nanofiber, as depicted in Fig. 5, followed by the deposition of the PVA/PAA onto the custom made of CuNi electrode on polyethylene terephthalate (PET) film. SEM images showed that they successfully immobilized the GOD onto the PVA/PAA nanofiber without aggregation. The functional group –OH interacted with the enzyme via hydrogen bonding with the –NH_2 terminal of the enzyme. The formed layer of PVA/PAA/GOD showed good conductivity based on low electrical resistance. In addition, electrospun PVA/PAA offered a well-defined porous structure of sub-micrometer size that blocked the white and red blood cells, in which the diameter ranged from 6 to 10 μm. Thus, only plasma that contained glucose can be passed through the pores and provide an unaffected current response against hematocrit concentration.

Cellulose is a highly chemical stable and biocompatible material that is widely used in *vitro* and *in vivo* biomedical applications [98]. Besides, β – Cyclodextrin (β – CD) is a non-toxic and biocompatible material with molecular structure of a hydrophobic core and a hydrophilic outer side that can efficiently trap small molecules such as protein or enzyme via host-guest mechanism [99]. To utilize these favorable characteristics of cellulose and β – CD for glucose sensing application, Kim et al. proposed a novel concept of cellulose/β – CD electrospun nanofiber for wearable epidermal glucose biosensors [100]. In this work, cellulose acetate (CA) and β – CD polymer mixture was electrospun on the reverse iontophoresis electrode. To convert cellulose acetate nanofiber into cellulose nanofiber, the diacetyl process was carried out with aqueous NaOH vapor. Eventually, cellulose/β – CD/GOx nanofiber was produced by the physical absorption of GOx on cellulose/β – CD/GOx nanofiber. The hydrophobic functional group of GOx is interlocked with the hydrophobic cavity of β – CD and well stabilized inside the nanofiber. Nanometric diameter (425 nm) of electrospun nanofiber conferred large surface areas that aid in absorbing moisture during the function of the sensor. Although the proposed design of cellulose/β – CD nanofiber is a novel idea for continuous glucose monitoring via reverse iontophoresis method, limit of detection was high and the sensitivity comparatively lower than other proposed designs.

Recent investigation has been done on the ability of direct electron transfer (DET)-based detection towards the enhancement and development of mediatorless biosensors. Mediatorless biosensors are sensors that do not have any extra reagents in a sample to detect the enzyme-substrate. DET occurs between the active redox enzymes and conductive nanomaterials, which plays an important role in developing electrochemical devices [101,102]. Enzymes immobilized on an electrode surface area capable of DET and retaining its bioactivity. However, several factors cause the difficulty of enzymes to carry out a direct electrochemical reaction. The first factor is the denaturation of the enzyme when they are adsorbed on the electrode surface, resulting in the loss of their electrochemical activities and bioactivities [6]. Secondly, the large three-dimensional structure of enzymes and the redox centers' resulting inaccessibility have made it complex to achieve the desired DET between enzymes and electrode surfaces. This further leads to the necessity to include promoters and mediators into the biosensors to obtain their electrochemical responses [6]. Zhang et al. proposed a DET-based
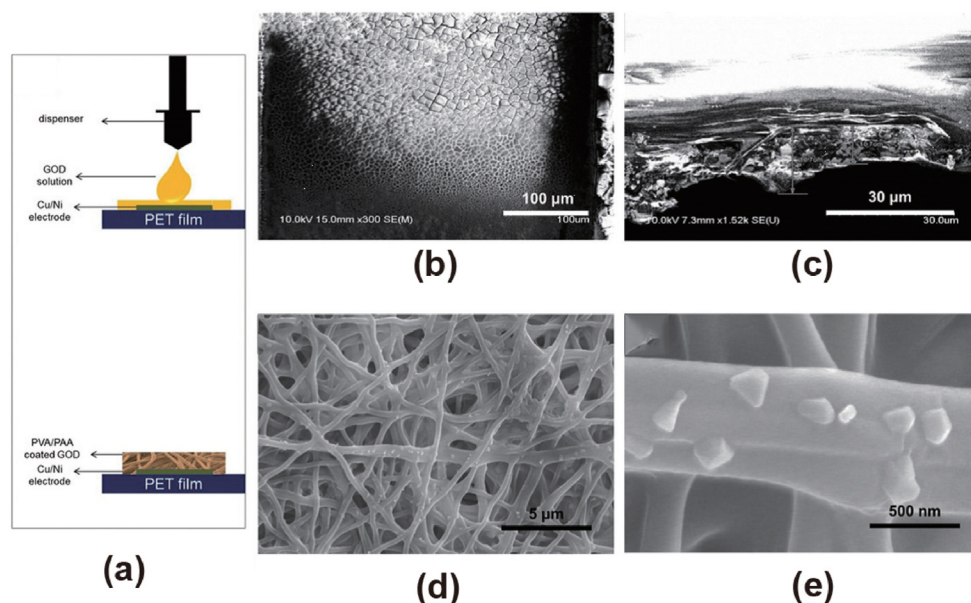

Fig. 5. Images of the fabricating process of the GOD dispensed on a Copper/Ni electrode (top) and PVA/PAA nanofibers coated GOD (bottom) [97].

glucose biosensor based on nitrogen-doped carbon nanospheres@carbon nanofibers (NCNSs@CNFs) composite [103]. The NCNSs@CNFs composites were prepared by the electrospinning of PPyNSs and PAN solution and, followed by the heat treatment and carbonization of PPyNSs@PAN nanofiber in the presence of N_2 gas. In the following step, NCNSs@CNFs thin film was cut and fixed on the glassy carbon electrode (GCE) electrode surface. Finally, GOx was coated on the modified electrode by the drop-casting method and Nafion was casted onto the NCNSs@CNFs/GOx-decorated electrode for retaining the stability. Prepared NCNSs@CNFs matrix was capable to be served as a substrate for GOx immobilization and realize the efficiency of DET of GOx without any pretreatment. The highly porous open structure of NCNSs@CNFs makes the DET between the active centers of GOx and the modified electrode easier and improve the mass diffusion of the matrixes. On the otherhand, this work showed that the denaturation of GOx upon adsorption onto the nanostructure surface was resulted by the change in its structure, and subsequently cause the loss of enzyme function. However, NCNSs@NFs serve as an ideal platform for the construction of the DET based sensors because of its ability to display moderated sensitivity with a very low detection limit whereas linear range was significantly wide [103].

Carbon-based electrospun nanomaterials serve as electron conductive material for biosensing and act as an excellent reinforced supporting material for wearable and implantable new generation biosensors due to their excellent mechanical properties. Notably, non-invasive wearable biosensors should possess certain properties such as high sensitivity, selectivity, ability to passage low current (<0.3 mA) across to the skin, fast response, and high stability to monitor glucose level continuously [104]. Electrospun polymer composite nanofiber matrix offers these features due to their high surface area to volume ratio, optimizable pore size, and engineered material composition. Recently, G.J. Kim et al. fabricated PVA/BTCA/β-CD/GOx/AuNPs hydrogel nanofiber substrate for wearable amperometric glucose sensor whereby non-invasive glucose monitoring can be carried out using interstitial fluid (ISF) [105]. In this study, separately synthesized GOx incorporated β-CD was mixed into the AuNPs – PVP polymer solution and, before synthesizing PVA/BTCA/β-CD/GOx/AuNPs hydrogel nanofiber, the resultant solution was electrospun and heated. Herein, (1,2,3,4-Butanetetracarboxylic acid) BTCA was added to the electrospinning precursor solution as a cross-linking agent. During the heating process, the carboxylic acid group of BTCA reacts with the hydroxyl group of PVA and β-CD to cause the esterification reaction. This novel hydrogel-based sensor matrix exhibited high sensitivity and low detection limit for especially, wearable sensor applications. Notably, this nanofiber matrix showed well-balanced mechanical properties (dried: 12.1 MPa, wetted: 5.33 MPa), high absorbency (DI water: 21.9, PBS: 41.91), and high enzyme activity of 76.3%.

3.2. Conductive-polymer based nanofibers for glucose detection

Conducting polymers such as polypyrrole (PPy), polyaniline (PANI), and poly (3,4-ethylene dioxythiophene) (PEDOT) are widely used for enzyme immobilization [106]. Incorporating enzymes into conducting polymer matrices provides stable biocatalyst interfaces and has broad and crucial practical applications. The embedment of enzymes into the conducting polymer film matrix maintains the accessibility of the catalytic sites while preventing the enzyme from leaching out [107]. Relevant studies show that the GOx enzyme loaded (surface coated or entrapped inside the nanofiber) electrospun conducting polymer nanofibers for glucose detection offers higher sensitivity and stability, leading to a longer lifetime compared with conducting polymer film counterparts [108,109]. By increasing the signal-to-noise ratio, electrospun conducting polymers serve as a suitable matrix for the immobilization and embedment of enzymes (bioelectric surfaces) [108].

It has been proven that the PEDOT conductive polymer matrix offers higher electrical conductivity and chemical stability for electrochemical biosensing applications [110]. The finding supported the investigation

that GOx post-entrapment in conducting polymers (PEDOT in this case) offers higher resistance towards the denaturation due to the changes in external factors, leading to functional retainment particles [111]. Recently, Yang et al. demonstrated a new and novel approach to fabricate GOx-incorporated-PEDOT coated microelectrode array for glucose detection, as shown in Fig. 7. PEDOT was directly synthesized on the Pt microelectrode surface by electropolymerization method, and GOx incorporated PEDOT was deposited on the electrode surface by electrodeposition (Fig. 7). Then, poly (L-lactide) (PLLA) nanofibers were electrospun on Pt microelectrode arrays followed by the electrochemical deposition of GOx incorporated-PEDOT polymer (PEDOT NFs-GOx) on the PLLA electrospun fiber surface. Optical and scanning electron microscopy (SEM) images of PEDOT F-GOx and PEDOT NFs-GOx on the Pt sides and Pt microelectrode arrays were shown in Fig. 6h and i. The designed sensor exhibited very low impedance and moderated sensitivity. Besides, the fabricated electrospun nanofiber is known for its high GOx enzyme entrapping [108].

Another work also proposed PEDOT-GOx nanofiber by introducing chemical vapor polymerization of PEDOT onto PAN nanofiber under an active vacuum condition at various times to form PEDOT, which later GOx solution was dropped onto the formed PEDOT [112]. Longer exposure periods formed higher amounts of PEDOT, which led to the blocking of PAN nanofibers (Fig. 8). An optimum polymerization period of 5 min was recommended where the mats showed the characteristic porous structure of PEDOT in a homogeneous nanofiber morphology loaded with five different enzyme units of GOx. The PEDOT-NFs/GOx nanofiber biosensor, i.e., loaded with 185 U GOx showed good sensitivity (74.22 μA mM^{-1} cm^{-2}) and LOD (2.9 μM) with a response time of 2–3 s without any interference while PEDOT-NFs/GOx-2 biosensor loaded with 92.5 U GOx exhibited extremely high sensitivity about 272.58 μA mM^{-1} cm^{-2}. However, all fabricated sensors of this work failed to have suitable operational stability.

3.3. Metal nanoparticle-based composite electrospun nanofibers for glucose detection

Gold nanoparticles (AuNPs) are proven to be a privileged metal for glucose oxidation. AuNPs can increase their catalytic activity toward glucose oxidation by depositing themselves onto the supporting matrix [113,114]. The deposition of metal NPs, with sizes, preferably in the nanometer range on the supporting materials, is crucial in preparing high-quality supported metal catalysts. Eleni et al. reported a novel and effective method to create nanostructure with a bioactive surface by assembling AuNPs into enzyme-based electrospun nanofibers for electrochemical biosensor applications [115]. They managed to develop PVA)/PEI/GOx nanofibers with gold nanoparticles assembled for ultrasensitive electrochemical glucose biosensing. First, the gold electrode was incubated with a 4-aminothiophenol (4-ATP) ethanolic solution for anchoring the 4-ATP layer at the surface of the electrode (Fig. 9). After that, the PVA/PEI solution was electrospun on the GOx modified electrode with minimal beading, taking into account the different parameters involving the applied voltage, the distance between nozzle tip and collector, and the solution feed. The self-assembled monolayer of 4-ATP possesses thiol groups for covalent binding onto the gold surface and mine groups to react with the amine groups of PEI nanofiber in the following cross-linking step using glutaraldehyde vapor. This leads to a better anchoring of NFs onto the electrode and enhanced water stability. Finally, AuNPs were decorated by dipping the PVA/PEI electrode into AuNPs colloidal solution. Decorating the NFs with AuNPs can attribute to the hydrogen bonding and ionic interactions between the carboxylic groups of citrate-stabilized AuNPs and the amine groups of PEI, leading to an enhanced conducting property. The impedimetric results imply that achieving a good linear response range and a very low detection limit. Furthermore, this sensor successfully preserved the enzyme activity and exhibited good stability [115].

Research has moved towards the usage of synergistic effects of two or
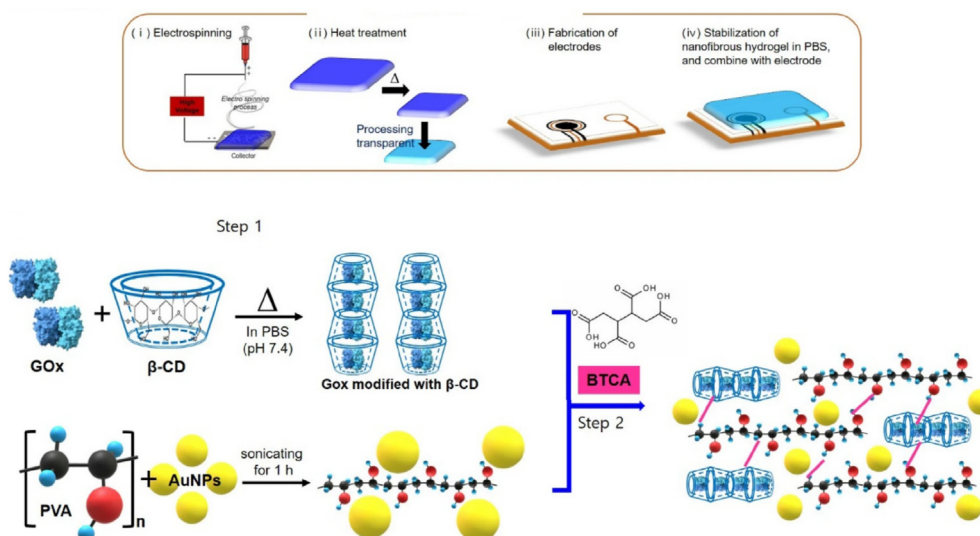
Fig. 6. Schematic illustration of the patch-type glucose sensor using PVA/BTCA/β-CD/GOx/AuNPs NF hydrogels on electrodes and the glucose-sensing mechanism for the non-invasive real-time monitoring of glucose in sweat. (b) Schematic illustration of the preparation of the PVA/BTCA/β-CD/GOx/AuNPs complex dope solution for electrospinning [105].

more than two components to improve the electrocatalytic, signal amplification, and sensing performance of biosensors. Particularly, noble metal nanoparticles hybridized with other metal or metal oxide composite materials exhibit excellent electrocatalytic and signal amplification properties for glucose sensor applications. Recently, Park et al. produced Cu nanoflower-decorated AuNPs – graphene oxide (GO) electrospun nanofiber matrix for glucose detection [116]. During the fabrication of the sensor matrix, initially, GO nanofiber was produced by the conventional electrospinning of PVA polymer and GO solution on the gold electrode. In order to deposit AuNPs and Cu nanoflowers on GO nanofiber, additionally prepared AuNPs and Cu nanoflower colloids were drop-cast on the electrospun fiber layer by layer as depicted in Fig. 10. Notably, the morphology of the electrospun nanofiber was determined by the GO concentration in the precursor solution. It was expected to achieve improved enzyme activity and stability through the large surface to volume ratio of Cu nanoflower decorated AuNPs nanostructure. Further study revealed that the Cu nanoflower decorated AuNPs – GO nanofibers produced higher cathodic current than the AuNPs – GO sensor, indicating that the electrocatalytic efficiency of the sensor matrix has been improved by the composite Cu nanoflower-decorated AuNPs - GO structure. In the presence of GOx, this sensor achieved a wide linear range of glucose concentration and a very low detection limit.

As another noble metal nanoparticle, Ag nanoparticles (AgNPs) possess excellent glucose oxidation and electrocatalytic properties [117]. A sensitive, non-enzymatic glucose sensor was fabricated by Zheng et al. using AgNPs/CuO nanofibers (NFs) [118]. Firstly, $AgNO_3$, $Cu(NO_3)_2$, and PVP were electrospun onto the surface of an indium tin oxide (ITO) glass, followed with oxidation to Ag/CuO NFs by annealing in the air to form the final Ag/CuO NFs-ITO electrode. Based on the amperometric analysis, it was found that the analytical performances in terms of sensitivity of the Ag/CuO NFs-ITO electrode are comparable or even better than other CuO nanomaterials or electrospun nanomaterials modified non-enzymatic glucose sensors. The results highlighted that the current response signal to glucose could retain more than 93% of its original response after being stored for one month, demonstrating the ability of long-term stability of the electrode. It is noted that electrochemical glucose biosensors can lose their activity due to the chloride poisoning effect [119]. However, Ag/CuO NFs-ITO electrode showed high selectivity for glucose detection in the presence of a high concentration of chloride. The finding concluded that the Ag/CuO NFs-ITO sensor can perform non-enzymatic glucose sensing with high sensitivity, low

nanometric detection limit (about 51.7 nM), excellent stability, and good reproducibility [118].

On the other hand, transition metal nanomaterials such as Co and Ni received considerable attention for glucose sensing due to their high electrocatalytic property [120]. However, the poor conductivity of these materials emerges as a major drawback for using this element alone for biosensing applications. As a solution, incorporation of metal nanoparticles or graphene nanomaterials with transition metals may confer great conductivity and electrocatalytic property for these materials. Recently, Li et al. investigated FeCo porous carbon nanofiber for non-enzymatic glucose sensing applications [121]. In this work, $Co(CH_3COO)_2$ and $Fe(CH_3COO)_2$ mixed PVP slurry was electrospun, and a 3D matrix was produced (Fig. 11). Following the procedure, FeCo porous carbon nanofiber was synthesized through the N_2-gas-involved high temperature (800–1000 °C) pyrolysis process. During the pyrolysis step, the formation of FeCo alloy and the degradation of the polymer matrix occurred. Also, pyrolysis temperature highly influenced FeCo carbon nanofiber's porosity, and high porosity was obtained at 800 °C. Finally, FeCo porous carbon nanofiber powder was dispersed into the Nafion solution and deposited on GCE electrode to obtain the optimized FeCo@PCNSs – 800 sensor matrix. This sensor yielded acceptable sensitivity and a lower detection limit. This study presents a simple processing method for synthesizing an alloy-based 3D sensor matrix with a mesoporous channel that increases mass transportation during the glucose oxidation reaction.

3.4. Electrospun metal oxide nanofibers for glucose detection

The substrate electrode selection is important as its material properties were proven to be influential on the faradaic current of glucose oxidation. It is reported that nanostructured one-dimensional metal oxide such as zinc oxide (ZnO), copper oxide (CuO), and nickel oxide (NiO) exhibit properties of large specific surface area, chemical stability, electrochemical activity, high electron mobility, and biocompatibility [122–124]. Several research groups have emphasized that electrospinning method is an effective technique for the fabrication of composite and inorganic nanofibers that play the role of glucose sensors [125–127]. The conventional way of synthesizing metal oxide nanofibers includes laser ablation and vapor-liquid-solid growth, which involves high energy, time, and cost. On the other hand, the electrospinning method was found to be an efficient method for the preparation of metal
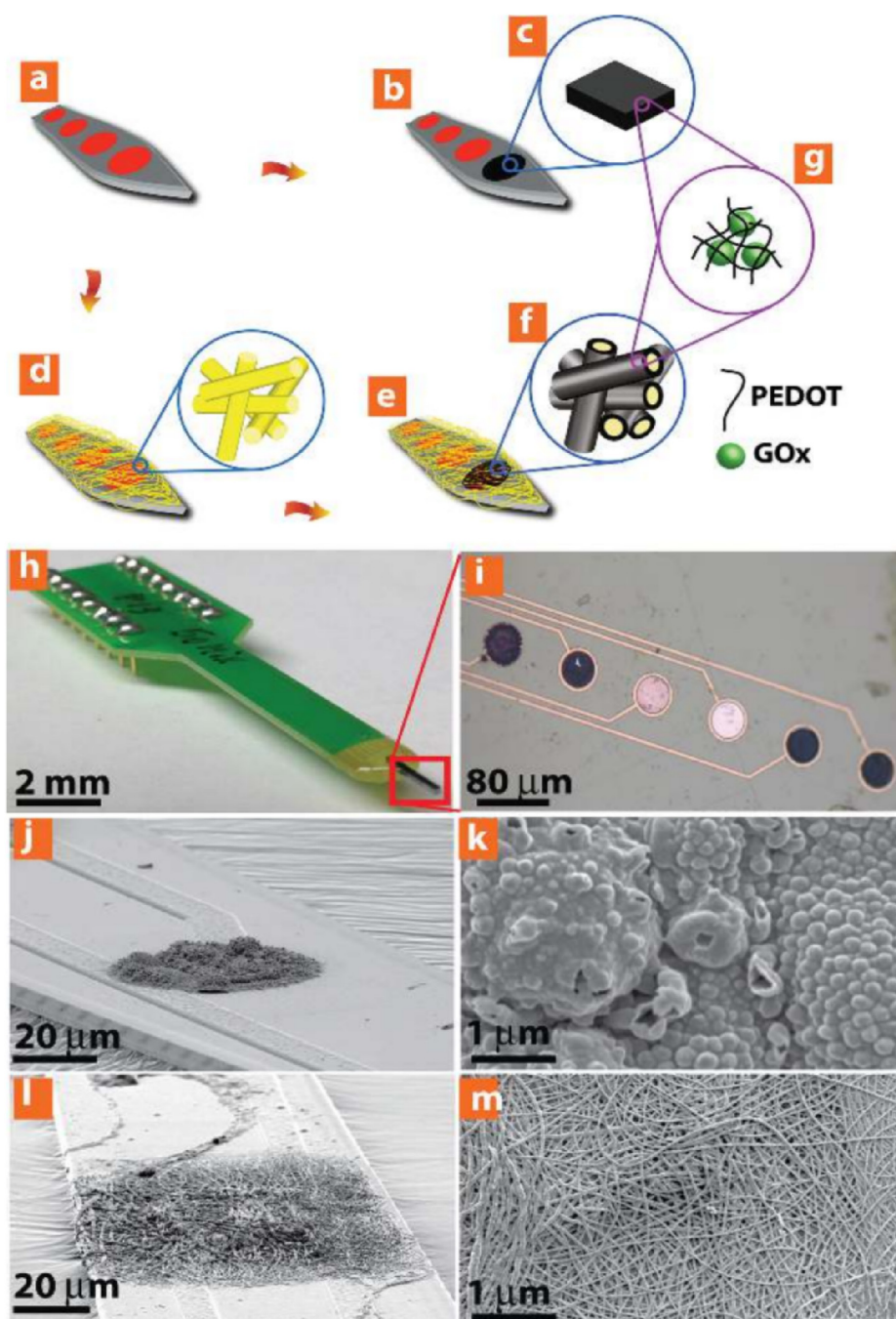
Fig. 7. Schematic of the fabrication process of GOx-incorporated PEDOT on the microelectrode array: a) Pt microelectrode array. b,c) electrodeposition of GOx-incorporated PEDOT film (PEDOT F-GOx). c) electrospinning of PLLA nanofibers on the microelectrode. g) entrapment of GOx within the PEDOT fiber. h) optical micrograph of the entire microelectrode array. i) optical micrograph of two uncoated Pt sites and four GOx-incorporated PEDOT sites of microfabricated electrodes. j) scanning electron micrograph of PEDOT F-GOx. k) SEM of PEDOT F-GOx. l) scanning electron micrograph of PEDOT NFs-GOx. m) SEM of PEDOT NFs-GOx [108].

oxide nanofibers as well [126,128]. Herein, calcination of electrospun metal nanoparticles or ion composite fibers with high temperatures is a commonly used approach in the fabrication of metal oxide nanofibers. The calcination process is used for the oxidative conversion of the organic phase in composite nanofibers. The morphology and properties of metal oxide nanofibers depend on the calcination temperature, heating rate, time, and the environment [126,128].

ZnO nanostructures are known as promising candidates for biosensing applications due to their distinctive features such as good electrical conductivity, high isoelectric point (IEP), non-toxicity, and inexpensiveness. Notably, ZnO nanofibers and nanowires show high electrocatalytic activity and efficient electron communication features as its high specific area. In a study done by Ahmad et al., a novel fabrication approach of a highly sensitive enzymatic glucose biosensor based on a single ZnO nanofiber was successfully demonstrated [127].

Electrospinning was used for the fabrication of nanofibers of poly (vinyl pyrrolidone)/zinc acetate composite. This is followed by high-temperature calcination of the above precursor fibers. Then, the individual ZnO nanofiber was transferred to a conventional gold electrode. Finally, the PVA solution is dropped onto the ZnO nanofiber/gold electrode to make sure that it is tightly attached to the surface of the gold electrode. It is then left to dry to form a film on the individual nanofibers. It is important to note that calcination, temperature, heating rate, and environment played crucial roles in determining the properties of the nanofibers. Nanoparticle formation was observed during the crystallization of the metal oxide in the calcination process of the composite nanofibers [129]. The mechanism of the sensing process involves the oxidation of glucose by GOx (OX) to gluconolactone, while GOx (OX) is reduced to form GOx(R). By the presence of oxygen in the solution, the consumed GOx (OX) could be generated from GOx(R), and hydrogen
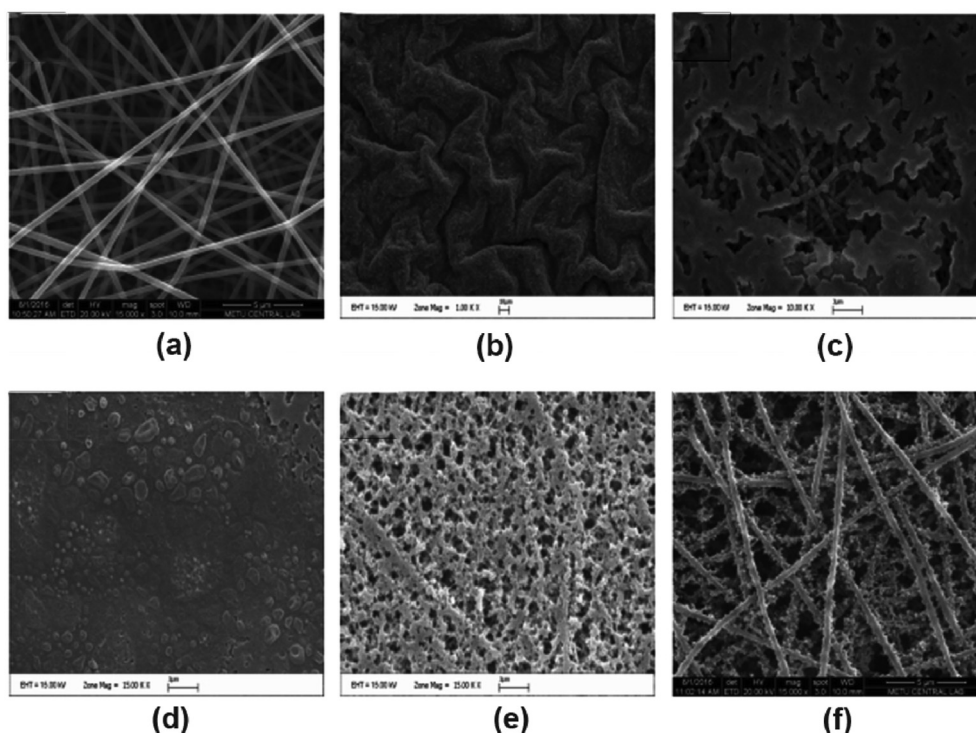
Fig. 8. SEM images of PAN nanofibers (a) and PEDOT nanofibers that were achieved after polymerization periods of (b) 24 h, (c) 3 h, (d) 15 min, (e) 10 min, and (f) 5 min [112].
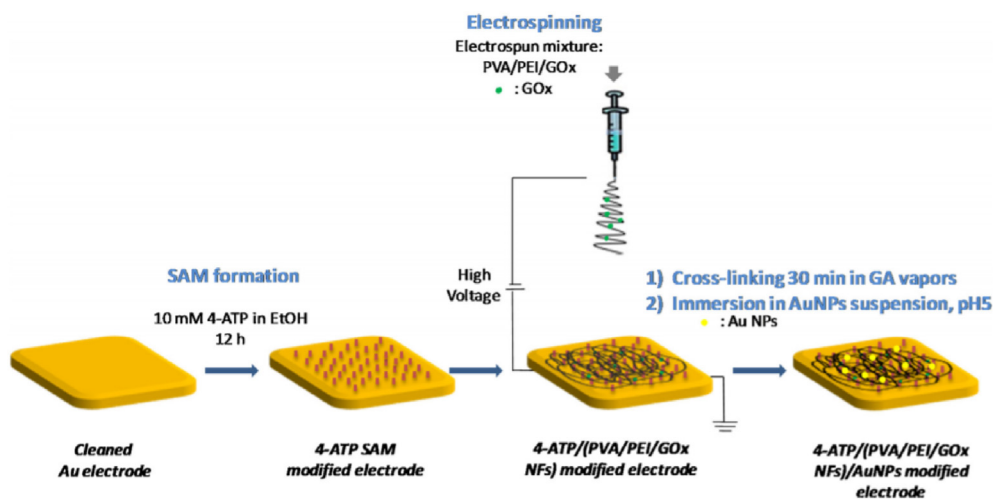

Fig. 9. Schematic illustration of the different steps involved in the fabrication of the proposed biosensor [115].

peroxide (H_2O_2) will subsequently be produced, which is used for the quantitative detection of the biosensor electrochemical capability on the modified electrode. Using cyclic voltammetric (CV) sweep curves, the bare and modified electrode without glucose was compared. It is found that there is a significant increase in the oxidation current, relating to the oxidation of glucose by GOx catalysis. Hence, ZnO nanofiber improves the electrocatalytic activity of the enzyme by enhancing the sensitivity of the biosensor for glucose detection. The performance of the biosensor showed good sensitivity and a low detection limit with a moderated response time around 4s. It also exhibits good anti-interference ability and good stability over long-term storage (more than 4 months) [127].

Various kinds of composite and alloy-based metal oxides have recently been investigated for non-enzymatic glucose monitoring due to the intrinsic drawbacks of insufficient stability and reasonable

repeatability in enzyme-based biosensors. Non-enzymatic electro-catalysts come in various forms, such as metals [130,131], metal oxides, bimetallic/alloys, carbon nanomaterials, metal/metal oxide heterogeneous nanocomposites, and metal/carbon nanomaterial-based composite layered double oxides [130]. The incorporation of metal nanoparticles into metal oxide nanofibers and bimetal oxide nanomaterials was found to enhance electrochemical properties. Notably, ZnO incorporated bimetal oxide nanofibers were found to be a well-known functional material in glucose biosensors due to their non-toxicity, stability, good biocompatibility and fast electron transferability between the active sites and electron [30]. Based on the study by Zhou et al. a three-dimensional (3D), porous ZnO–CuO hierarchical nanocomposites (HNCs) glucose electrodes can be fabricated with different thicknesses [132]. In this study, ZnO–CuO hierarchical nanocomposite fiber was synthesized using
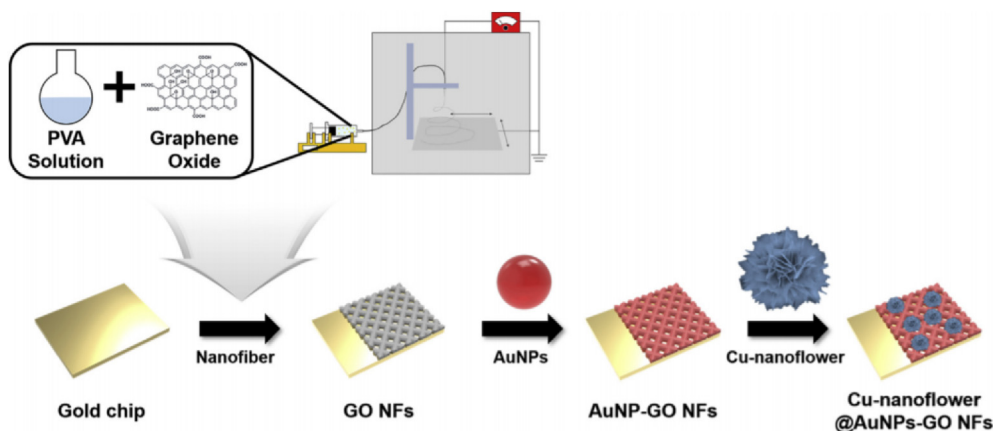
Fig. 10. Schematic illustration for the fabrication of Cu-nanoflower - AuNPs-GO nanofibers (NFs)-based electrochemical glucose nano-biosensor [116].
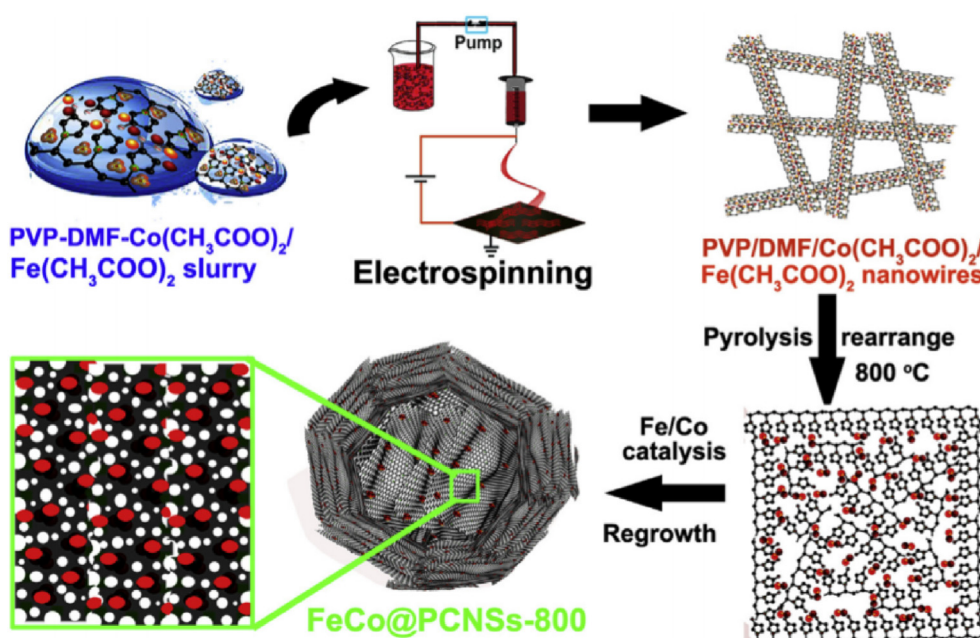

Fig. 11. Schematic illustration for the preparation procedures of the 3D hierarchically porous FeCo@PCNSs – 800 sensor matrix [121].

coaxial electrospinning followed by hot press and calcination. In this process, zinc acetate mixed PVP slurry (outer solution) and copper acetate mixed PVP slurry (core solution) were electrospun directly onto the FTO (Fluorine doped tin Oxide) electrode. Finally, the electrospun electrode was calcined at 500 °C to remove the polymer matrix. The results showed that the sensing properties of 3D porous ZnO–CuO nanofiber coated electrodes were greatly enhanced and strongly depend on the coating thickness. The report concluded that the formation of special hierarchical heterojunction and well-constructed 3D structure of ZnO–CuO nanofiber contributed to the outstanding long-term stability, good reproducibility, very high sensitivity around 3066.4 $\mu AmM^{-1}cm^{-2}$, low detection limit, linear detection range, good selectivity, and accurate measurement of the biosensors [132].

CuO, a p-type metal oxide semiconductor with a narrow bandgap (1.2eV), is known for its unique characteristics and nanostructures that can be successfully used for optical and electrochemical applications. For instance, it has been reported that varying pH of the solution leads to a change in the shapes of CuO nanostructures [133]. Mainly, high specific area, chemical stability, electrochemical activity, and high electron conductivity features of CuO nanofibers confer excellent sensing performance for glucose sensing applications. In addition, it possesses

exceptional electrochemical activity and is capable of promoting electron transfer at a low potential that improves the sensitivity of glucose sensors at low detection limits [73,134,135]. In a recent work, detection of glucose in saliva was successfully conducted using non-enzymatic biosensor of CuO nanoparticles and polycaprolactone@polypyrrole fibers on a modified indium-tin-oxide (denoted as CuO/PCL@PPy/ITO) electrode through electrospinning and electrodeposition method (Fig. 12) [136]. In this study, Polycaprolactone (PCL) was electrospun on ITO glass. The electrospun PCL fiber was dipped into polypyrrole (PPy) prior to the electrodeposition of Cu nanoparticles with applied potentials between −0.8 and −1.4 V at various concentrations of copper sulfate (5–20 mM). Fig. 12 confirms the core-shell structure of electrospun nanofiber and Cu nanoparticles were well dispersed, and the active surface area has been increased dramatically. The prepared CuO/PCL@PPy/ITO electrode was tested with glucose-containing saliva. The decorated CuO nanoparticles enhanced the conductivity of the electrode and improved the detection of glucose via transformation of the Cu redox couples from Cu(II) to Cu(III). In saliva samples, quantitative detection of glucose is determined in the linear range from 2 µM to 6 mM, and the lowest detection limit was 0.8 µM.

High conductive band of CuO is one of the main issues in
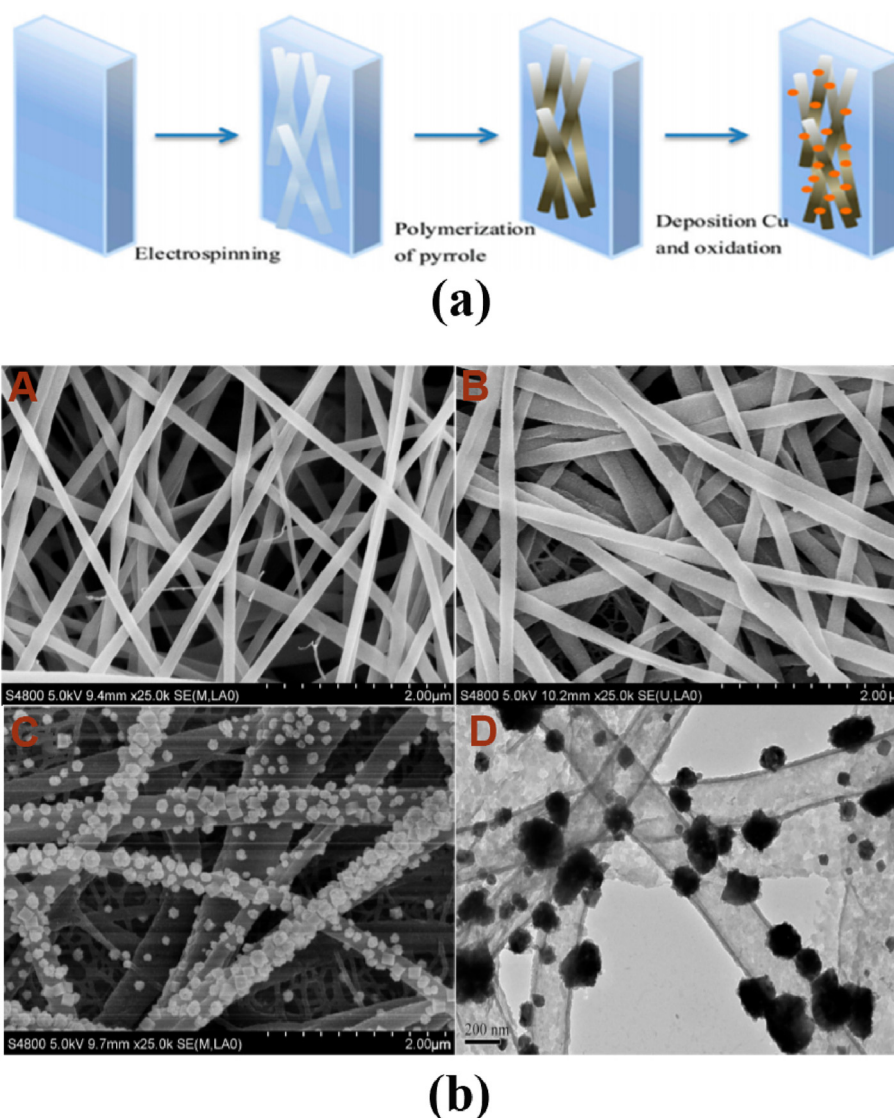
Fig. 12. (a) Schematic of fabrication of CuO/PCL@PPy on indium-tin-oxide (ITO) electrodes. (b) FE-SEM images of (A) PCL nanofibers; (B) PCL@PPy; (C) CuO/PCL@PPy; (D) TEM image of CuO/PCL@PPy [136].

electrocatalytic applications. As a solution, nitrogen doping is commonly applied to introduce nitrogen species into the carbon matrix, enhancing the electron density and changes the electron donor/acceptor properties to improve the electrocatalytic and conductivity performances [137–139]. Alternatively, depositing CuO on the nitrogen-enriched carbon supports, such as electrospun CuO composite nanofibers can be used. Moreover, electrospun carbon nanofibers (CNFs) possess a large aspect ratio and three-dimensional porous networks, which enhance the electron transfer rate and becoming robust support for CuO deposition [140]. Also, the ultra-long one-dimensional nanostructure of s carbon nanofiber can reduce the charge diffusion length and enhance the accessible surface area of CuO with electrolyte, improving the electrocatalytic activity [141]. Lu et al. successfully demonstrated CuO nanoparticles/nitrogen-doped carbon nanofibers modified glassy carbon electrodes for non-enzymatic glucose sensors [142]. In this report, polyacrylonitrile (PAN) and in-situ polymerization using aniline monomers were used to obtain polyacrylonitrile@polyaniline core-shell nanofibers (PAN@PANI nanofibers). Next, in-situ nitrogen-doping through carbonizing PAN@PANI nanofibers was used to prepare N-CNFs. It is reported that the produced N-CNFs possessed higher nitrogen content compared with CNFs arising from pure PAN. The process is then

followed by a facile solvothermal reaction where CuO NPs were used to decorate on N-CNFs (CuO/N-CNFs). The report shows that the 3D hierarchically porous structure of N-CNFs enhanced the electrochemical reactions by providing ion transport channels and facilitate large mass transport. CuO/N-CNFs are then modified on glassy carbon electrodes (GCE) to obtain CuO/CNFs/GCE. The results showed that CuO/N-CNFs/GCE showed the best performance for glucose detection comparing with CuO/CNFs/GCE and pure CuO/NPs/GCE. It demonstrated good sensitivity, moderated detection limit and good selectivity in the presence of general interferences in blood, and high stability with long-term exposure in the air [142].

Ni-based nanomaterials have been widely used to construct electrochemical sensors due to their excellent electrocatalytic properties [143] and stability [144]. Chen et al. combined electrospun carbon nanofibers (ECFs) and Ni(OH)$_2$ nanoplatelets to construct a highly sensitive non-enzymatic glucose sensor [145]. Fabrication of the Ni(OH)$_2$/ECF hybrid was conducted by producing ECF film followed by carbonization (Fig. 13). Initially, PAN nanofibers were synthesized by electrospinning, followed by oxidation and carbonization of the PAN electrospun nanofibers through high-temperature calcination to produce carbon nanofibers. Afterwards, the Ni(OH)$_2$ nanoplatelets on the ECFs were prepared
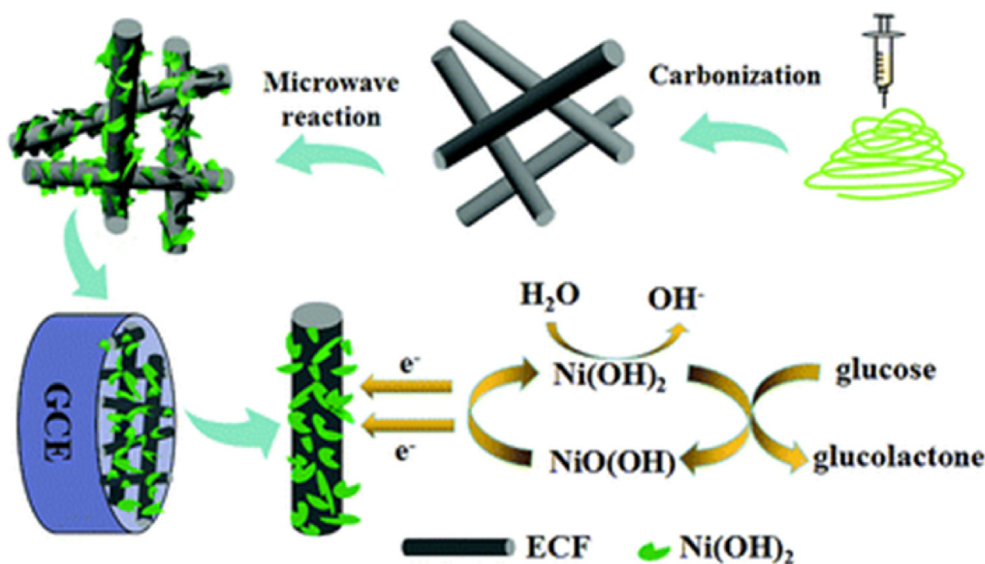
Fig. 13. Construction procedure for Ni(OH)2/ECF sensor [145].

by soaking the ECFs into a different concentration of Ni(NO$_3$)$_2$·6H$_2$O and urea solution and heated under microwave irradiation. Finally, the produced Ni(OH)$_2$/ECFs were dispersed in a mixture of Nafion by sonication and placed onto the glassy carbon electrode by casting. The fabrication method is depicted in Fig. 13. It was observed that the catalytic currents of Ni(OH)$_2$/ECF-modified electrodes were markedly larger than that of the pure Ni(OH)$_2$ nanoplatelet-modified electrode, indicating enhanced electrocatalytic activity for the Ni(OH)$_2$/ECF hybrids. This might be attributed to the synergistic effect of well-dispersed Ni(OH)$_2$ and ECF, and the increased hybrid conductivity and surface area. The fabricated Ni(OH)$_2$/ECF showed high sensitivity and a very low detection limit with a linear detection range.

GO/rGO nanomaterials incorporated NiO composite nanomaterials received considerable attention for glucose sensor applications due to their excellent electrical conductivity, high chemical and thermal stability [146]. Considering these facts, Zhang et al. fabricated GO-incorporated NiO$_2$-electrospun-fiber-coated electrodes for non-enzymatic glucose sensing applications [147]. In this work, the NiO$_2$

nanofiber matrix was prepared using Ni(II)acetate-PVP solution followed by high-temperature calcination. Next, GO suspension was transferred onto the GCE electrode by the self-assembly method to modify the electrode. After that, NiO$_2$ nanofiber suspension was transferred onto the GO-coated electrode by the drop-casting. Finally, the NiO$_2$/GO modified electrode was coated by Nafion for improving stability and avoid fouling. This sensor exhibits high sensitivity and low detection limit. However, comparatively this sensor took a long response time, which was about 5s. Furthermore, the layer-by-layer assembly of the process method can be difficult and affect the accuracy of the sensor.

Nanoneedle arrays of NiCo$_2$O$_4$ on a network of ECF membrane (NiCo$_2$O$_4$/ECF) was developed for non-enzymatic glucose biosensor on glassy carbon electrode (GCE) [148]. In this work, a free-standing ECF membrane was facilely obtained via electrospinning of PAN followed by the calcination process (Fig. 14). The carbonized PAN nanofiber was transformed into 3D open architecture by a facile one-pot hydrothermal reaction via autoclave at 120 °C in the solution that consists of Co(N-O$_3$)$_2$·6H$_2$O, Ni(NO$_3$)$_2$·6H$_2$O, urea, and deionized water. The 3D open

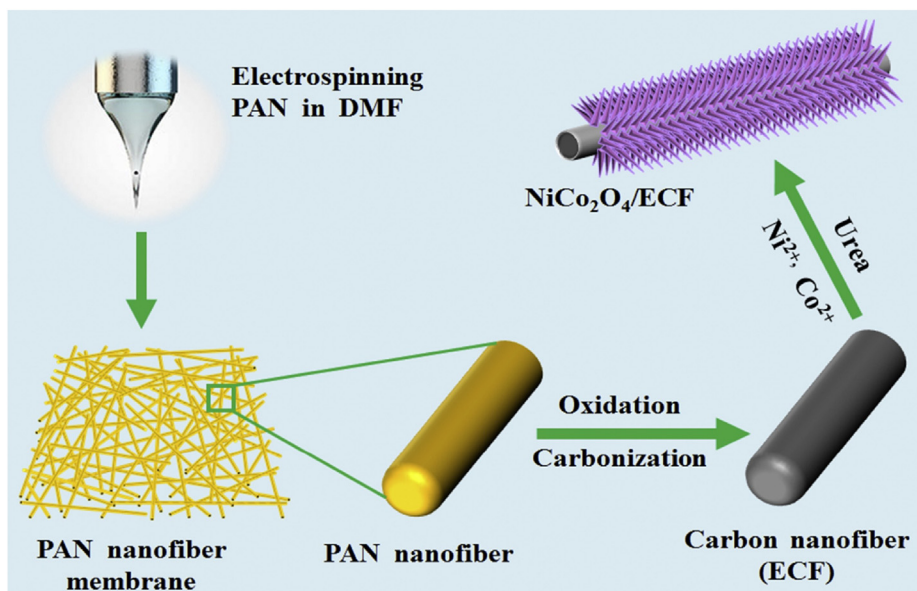**Fig. 14.** The fabrication procedure of NiCo2O4/ECF nanohybrids [148].

structure could serve as a high-surface-area template for further growth of electrochemically active materials with average ECFs diameter of 320–400 nm, and the length can reach tens of micrometer. However, the $NiCo_2O_4$/ECF hybrids present a wide linear range, low detection, and high sensitivity for glucose detection.

The metal oxides are not only used as an electrocatalytic agent for glucose sensing but can also be used as a scaffold for immobilizing glucose oxidase [149]. According to the proposed design, mesostructured and porous TiO_2 hollow nanofibers act as scaffolds that adsorb both enzyme and protein. In this study, a combination of electrospinning technology and sol-gel methods were employed to fabricate TiO_2 hollow nanofibers. Initially, an electrospun PAN nanofiber as template was dipped into the TiO_2 sol-gel whereby a thin layer of TiO_2 encapsulated onto the surface of PAN nanofiber (Fig. 15a). After calcining the TiO_2 – PAN composite at 550 °C, the free-standing hollow nanofiber TiO_2 structure was obtained (Fig. 15b). TEM and HRTEM images confirmed that the porously hollow nature of TiO_2 nanofiber was formed after calcination. Herein, the produced hollow nanofiber TiO_2 was used to immobilize the GOx. The highly porous surface of the hollow TiO_2 nanofiber further improved the electrocatalytic activity and electron diffusion rate that improve the performance of the sensor. The response of glucose oxidation on glass carbon electrode (GCE) with hollow TiO_2 nanofiber showed a moderated sensitivity and a very low detection limit.

Mixed valence oxides of transition metals with a spinel structure possess impressive magnetic, electrical, optical, and catalytic properties [150,151]. As depicted in Fig. 16, the spinel structure has empty sites in the interstitial space of the structure, allowing them to improve electron transfer [152]. This structure also possesses great flexibility in hosting various metal ions and reciprocal substitution between two sublattices, which promotes the magnetic and electrical properties of the spinel [153]. Electrospinning is one of the simplest and cheapest techniques for synthesizing spinel nanofibers [154]. Zhang et al. demonstrated the synthesis of $MnCo_2O_4$ nanofibers by electrospinning $Mn(Ac)_2$/PVP precursor solution, followed by calcination to fabricate a highly sensitive non-enzymatic glucose sensor [155]. During the electrode modification process, $MnCo_2O_4$ nanofibers with a glassy carbon electrode ($MnCo_2O_4$/GCE) were fabricated by simply drop-casting the $MnCo_2O_4$ electrospun fiber suspension on the GC electrode. As shown in Fig. 16, $MnCo_2O_4$ nanofibers consist of many nanoparticles with large surface areas, of which Mn and Co elements can provide abundant electrocatalytically active sites for the oxidation of glucose. The synthesized $MnCo_2O_4$/GCE electrode demonstrated excellent electrocatalytic ability, low detection limit, high sensitivity, wide linear range, and high reproducibility. The long-term stability of $MnCo_2O_4$/GCE was studied for 30 consecutive days and tested for every 5 days interval. The sensor successfully retained above 90% of its original counterpart. An interference test was carried out for the sensors, using easily oxidizable species such as uric acid (UA), dopamine (DA), ascorbic acid (AA), tryptophan (Trp), and glycine (Gly). The results showed that $MnCo_2O$/GCE possesses high selectivity for glucose detection. The sensor was also applied to
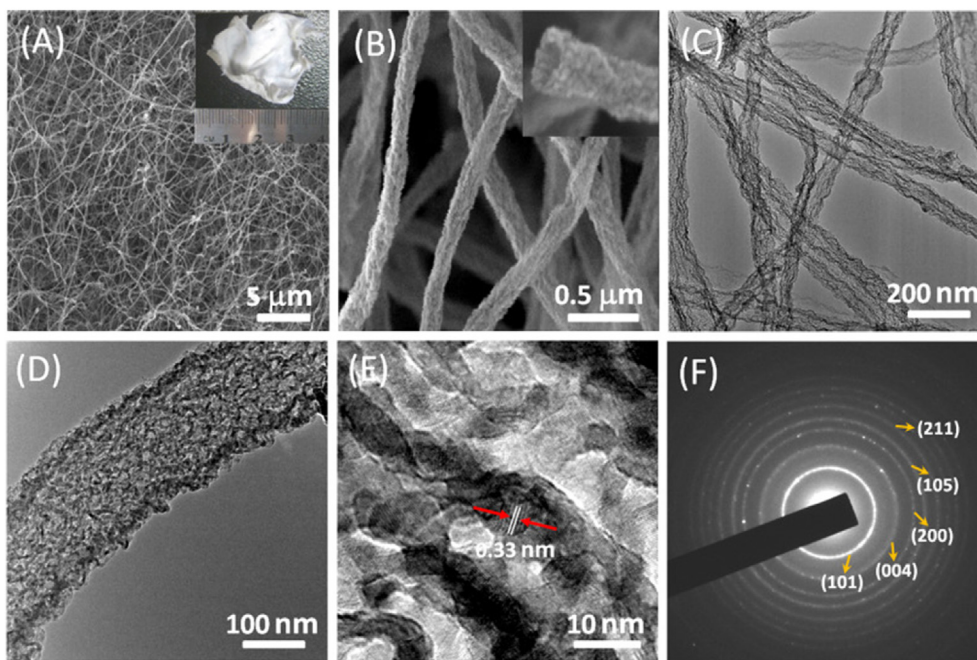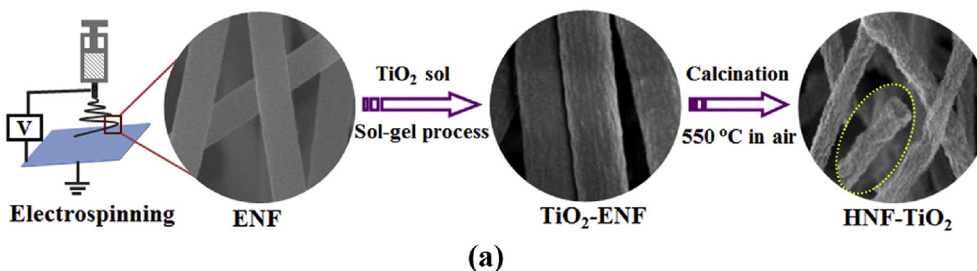
Fig. 15. (a) Schematic illustration for the preparation of TiO2 hollow nanofiber. (b) SEM (A, B), TEM (C, D), HRTEM (E) images, and (F) SAED pattern of HNF–TiO2. Inset of (A) and (B) are the optical photo and magnified SEM images of HNF–TiO2, respectively [149].
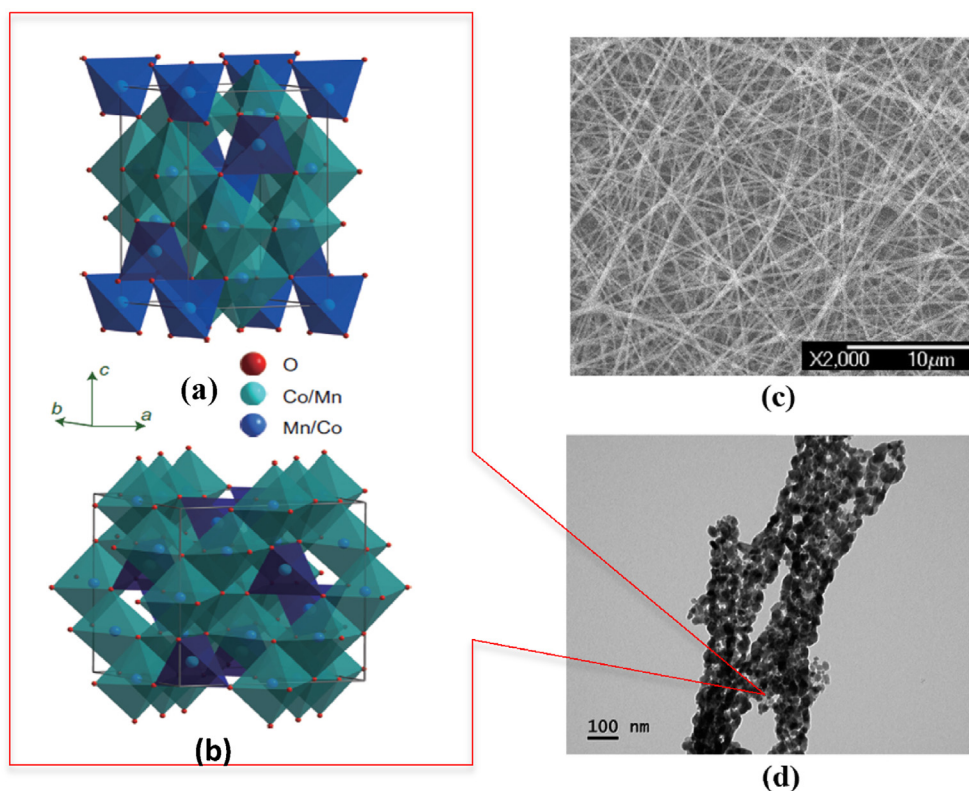
Fig. 16. Schematic representation of tetragonal (A) and cubic (B) spinels structures of MnCo2O4 crystals [174] and, (C) and (D) TEM and HRTEM images of MnCo2O4 electrospun fibers respectively [155].

determine glucose in human blood samples, and the results show that the sensor can recover 97.45–100% of the sample, concluding that it is indeed feasible as a glucose biosensor [155].

Rhodium oxide nanocorals (Rh_2O_3 NCs) were synthesized through $RhCl_3$/PVP nanofibers, followed by high-temperature calcination at 700 °C [156]. The calcined nanocorals (NCs) were mixed with ethanol and sonicated to produce Rh_2O_3 suspension, dropped onto a glassy carbon electrode (GCE) and coated with Nafion to form Rh_2O_3 NCs/Nafion/GCE electrode. As shown in Fig. 17 calcined electrospun nanofibers appear discrete coral shape which can afford huge active surface area for the sensor. The metal oxide-based glucose oxidation showed that metal oxide on the electrode reacts with OH- to release electrons and generate metal

oxide hydroxide followed by glucose oxidation and metal oxide regeneration. The fabricated electrode also showed a good response towards glucose in human serum. These features indicate that the as-prepared Rh_2O_3 NCs hold great promise and demonstrate a good electrocatalytic activity toward glucose oxidation in an alkaline medium. This sensor exhibits a lower detection limit of 3.1 μM, and a reasonable selectivity against various interferents in non-enzymatic glucose detection. However, the sensitivity of this material was comparatively less, around 11.46 μA mM−1 cm−2. Other separate work used a similar procedure to fabricate IrO_2 nanofibers-Nafion/GCE electrode with the same intention in detecting glucose [157]. The fabricated electrode showed a sensitivity of 22.22 μA mM^{-1} cm^{-2}, a limit of detection of 2.9 μM, and good
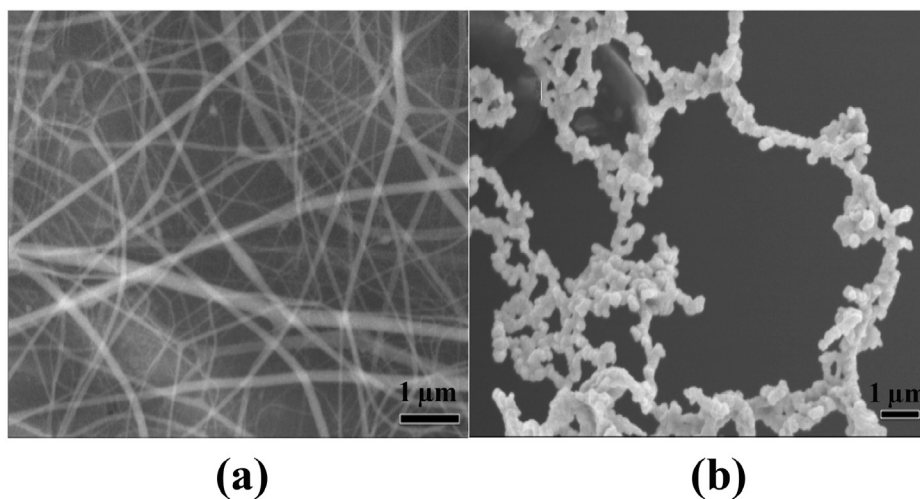
Fig. 17. Typical SEM images of (a) electrospun RhCl3/PVP precursory nanofibers and (b) Rh2O3 nanocorals after calcination of electrospun RhCl3/PVP precursory nanofibers at 700 °C for 3 h [156].

selectivity against various interference in non-enzymatic glucose detection.

3.5. Transition metal carbide nanofibers for glucose detection

Transition metal oxides are popular for glucose sensing applications due to their excellent catalytic activity. Similarly, nowadays, transition metal carbides are given more attention for electrochemical applications. Generally, transition metal carbides exhibit surface properties and catalytic attributes similar to noble metal nanoparticles. However, these transition metal carbides are extremely cheaper than noble metal nanoparticles [158–160]. Besides, selectivity, thermal stability, and high electrical conductivity of transition metal carbides make them a reliable candidate for glucose detection applications. Despite the advantages, transition metal carbides possess a low surface charge that resulting agglomeration of particles. However, this drawback can be overcome by electrospinning technology. Recently, Q. Guo et al. fabricated titanium carbide (TiC) – carbon nanofiber was prepared by the conventional electrospinning of TiCl$_4$, PVP, and PAN polymer mixture (Fig. 18) [160]. After that, electrospun nanofiber was pre-oxidized at medium temperature (200°) in air and followed by the carbonization at high temperature (1000 °C) in a vacuum environment. During the electrode preparation, TCNFs were initially dispersed into a solvent that consists nafion solutions. Finally, GOD mixed nanofiber dispersion was drop cast on GC electrode. Herein, the combination of titanium carbide with carbon nanofiber offered high surface area, low impedance, and improved electrocatalytic ability. Subsequently, this sensor exhibited high sensitivity, moderated detection limit, and a wide linear detection range.

4. Colorimetric detection

Generally, colorimetric glucose sensors consist of various reactants, such as enzymes, coenzymes, and chromogenic agents. However, conjugation of these elements on bio-receptors and carriers is considered a major challenge because they are not stable on particular receptors. Although various types of solid surfaces were introduced to overcome this shortcoming, the adsorption rate of these elements on the solid surfaces is rather poor. To addressing this problem, Ji et al. fabricated a hollow nanofiber-based colorimetric sensor that encapsulates the chromogenic agents and enzymes [161]. In this work, glucose oxidase (GOx), horseradish peroxidase (HRP), and chromogenic agents (2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS) or o-dianisidine) were used for glucose colorimetric detection in the bi-enzyme system. Coaxial electrospinning was used to prepare the hollow nanofiber membrane-based testing strips (Fig. 19 (a) and (b)). During the process, the shell phase precursor solution comprised of Polyurethane (PU) polymer mixed o-dianisidine and the core phase precursor solution comprised of GOx enzyme, HRP enzyme, and ABTS chromogenic agent with solvent. In the final product, GOx + HRP + ABTS encapsulated and o-dianisidine implanted hollow PU nanofiber was obtained. The prepared electrospun hollow nanofiber membrane was immersed into

different concentrations of glucose solution to detect glucose, and the optical detection of glucose was carried out by measuring the color intensity changes on the testing strip. As a result, the color intensity of the electrospun hollow fiber membrane increased with increasing concentration of glucose. This sensor delivers a low detection limit, around 0.01 mM, and a broad detection range from 0.01 to 20 mM. In addition, this sensor exhibited excellent long-term storage stability (stable for at least four months). This facile electrospinning approach and the advantages of the simultaneous in situ co-immobilization aid in developing various multienzymes, coenzymes or chromogenic agents based biosensors [161].

Luminescent transition metal complexes have been widely used for fabricating optical oxygen biosensors and glucose biosensors due to their strong photostability, large stokes shift, high quantum efficiency, and high oxygen quenching efficiency [162]. A sensitive and fast glucose biosensor using iridium complex-doped electrospun optical fibrous membrane was developed by Zhou et al. [162]. Iridium (III) bis (2-phenylbenzothiozolato-N,C^2) acetylacetonate ((bt) 2Ir (acac), was used as the luminescence probe instead of a reported ruthenium complex due to its higher quantum efficiency [162]. A one-step electrospinning technique functionalized with glucose oxidases (GOD/EOF) was used to fabricate polystyrene optical fibrous membrane (EOF) biosensor (Fig. 20). This fabrication approach contained both a recognition probe and a sensitive luminescence probe. The electrospinning of polystyrene (PS) polymer solution with (bt)2Ir (acac) mixture was prioritized as the first step with N, N′-dimethylformamide (DMF) as the solvent for (bt)2Ir (acac), (bt)2Ir (acac) molecule. DMF has good compatibility and dispersion capability and could stably and uniformly doped within the PS matrix to avoid the self-quenching and leaching effects during the luminescent measurements. Covalent immobilization of GOD onto the surface of EOF by using UV irradiation was done to prepare glucose oxidase (GOD)/EOF, followed by glutaraldehyde cross-linking for the use of glucose detection. The luminescence from EOF might be sensitive to dissolved oxygen at low concentrations due to the high oxygen quenching efficiency of (bt)2Ir (acac). It was found that luminescence of the GOD/EOF was fast and highly enhanced due to the fast electron or energy transfer between the EOF and dissolved oxygen was reduced. Thus, GOD/EOF can detect glucose quickly, sensitively, and specifically. It is also shown that GOD/EOF irradiation has a more luminescence intensity increase, comparing to that without irradiation at the same glucose concentration. It was proved that these irradiated PS fibers could be used as an effective biosensor support matrix for fabricating biosensors. Fig. 20 illustrates the quick detection of glucose by GOD/EOF. The report concluded that the enhanced detection performance of the GOD/EOF biosensor was attributed to its high surface-area-to-volume ratio, the efficient electron transfer between the optical fibers and dissolved oxygen molecules [162].

Quantitative detection of glucose using a competitive binding mechanism is considered as a prime and highly selective technique for glucose sensing. Subsequently, Yoon and Czarnik reported that boronic acids could bind to glucose irreversibly under physiological conditions.
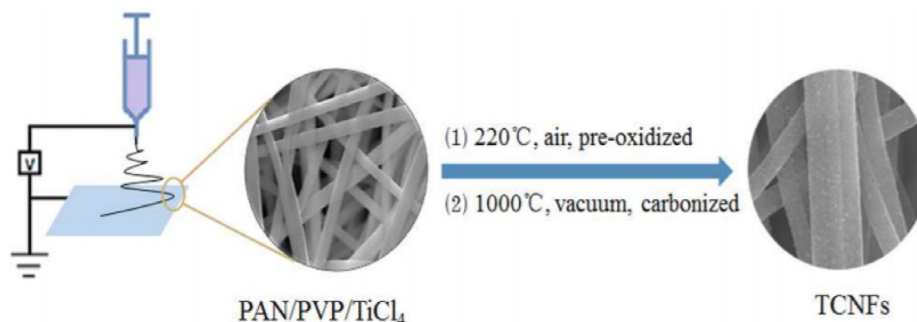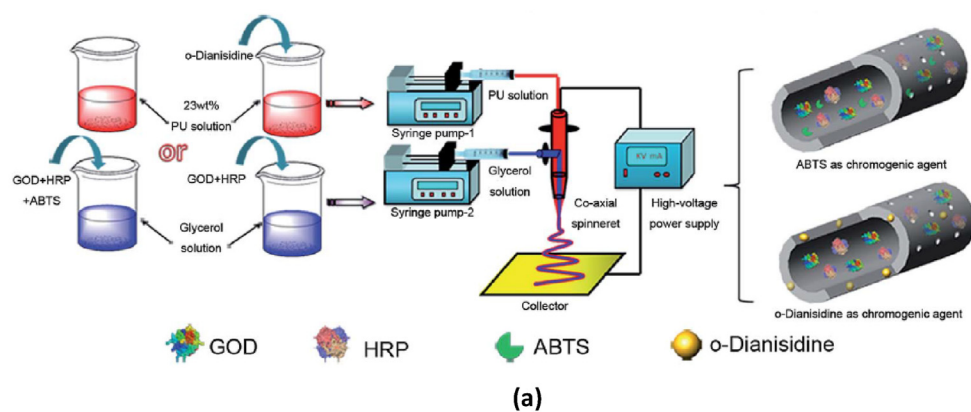

Fig. 18. Schematic illustration for the preparation of TCNFs [160].

Fig. 19. (a). Schematic illustrations of the bi-enzyme reaction for glucose measurement and the setup for co-axial electrospinning to prepare hollow nanofiber membrane-based glucose testing strips. During co-axial electrospinning, glucose oxidase (GOx), horseradish peroxidase (HRP), and chromogenic agent (ABTS or odianisidine) were simultaneously immobilized in situ in the hollow nanofiber membrane [158]. (b). Characterization of hollow nanofibers prepared by co-axial electrospinning. (A and B) Cross-sectional SEM images, (C) TEM images [161].
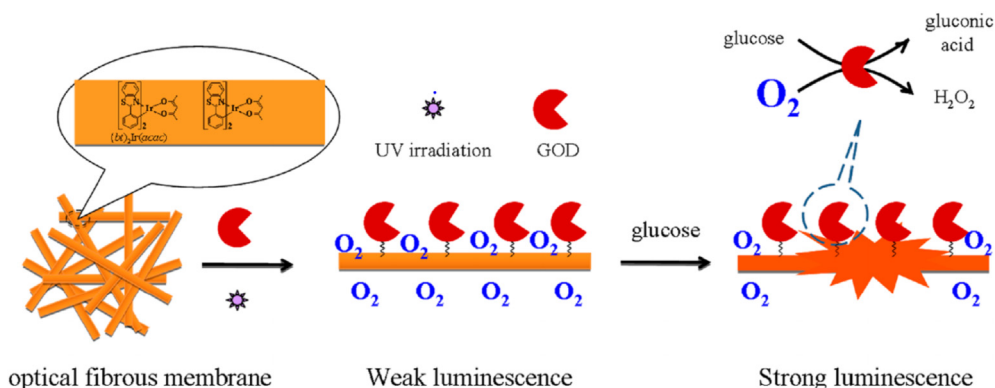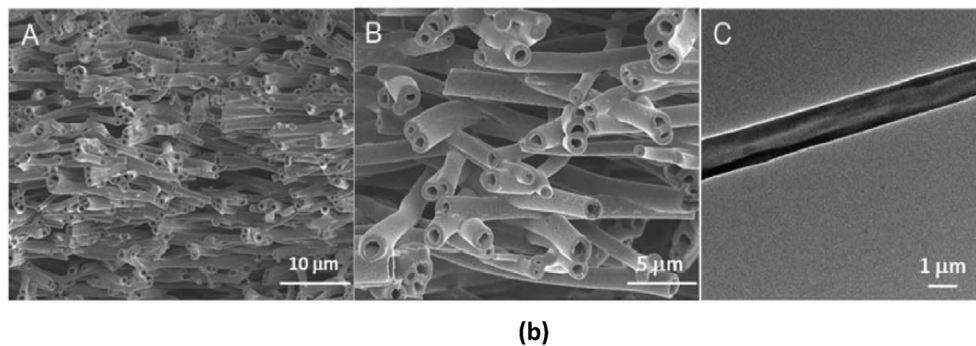
Fig. 20. Schematic Illustration of the rapid GOD/EOF glucose detection [162].

This binding exhibits a measurable change in fluorescent intensity using anthracenyl boronic acid as shown in Fig. 21 [163,164]. Considering these facts, Balaconis et al. developed a glucose-sensitive electrospun nanofiber decorated optode sensor [165]. In this study, the electrospun nanofiber-coated optode was synthesized using polycaprolactone (PCL) polymer, tridodecylmethylammonium chloride (TDMAC) polymer, citroflex plasticizer, alizarine dye, and boronic acid (BA) derivative (BA2b – BA2c) mixture. In this study, prior to the *in vivo* analysis, a 6 mm diameter biopsy punched nanofiber sensor scaffold was injected using an indwelling needle assembly to the back of mice for continuous glucose monitoring. This is to design an optode-based nanosensor that responds better to physiological glucose with higher stability at the site of *in vivo* implantation. Optode-based nanosensors react with physiological glucose concentration by producing hydrophobic boronic acids with electron-withdrawing groups in a competitive binding interaction between diols and boronic acids on either glucose or alizarin. In the absence of glucose, boronic acids will bind to diol on alizarin and statically quenches the fluorescence. In the case where glucose concentration is increased, glucose will displace alizarin and results in fluorescence. In this study, implantation site signal loss of spherical sensor and the electrospun sensor was compared with their *in vitro* signal loss. A higher signal loss was observed in spherical nanosensor at the implantation site compared to *in vitro* electrospun nanofiber sensor. *In vitro* signal loss is caused by the leaching of boronic acid from the hydrophobic core and the difference in signal loss between *in vivo* and *in vitro* is due to the diffusion of nanosensors away from the implantation site, and nanofiber scaffolds showed a very closely matched signal loss between *in vitro* and *in vivo*. Electrospun nanofiber sensor showed high stability, cellular uptake, and ability to retain the sensor components (either boronic acid or alizarin) in *vivo* condition that results in minimum signal loss. Besides, *in vivo* nanofibers retained sensor residency at the implantation site and are allowed longer monitoring times [165].

5. Summary and discussion

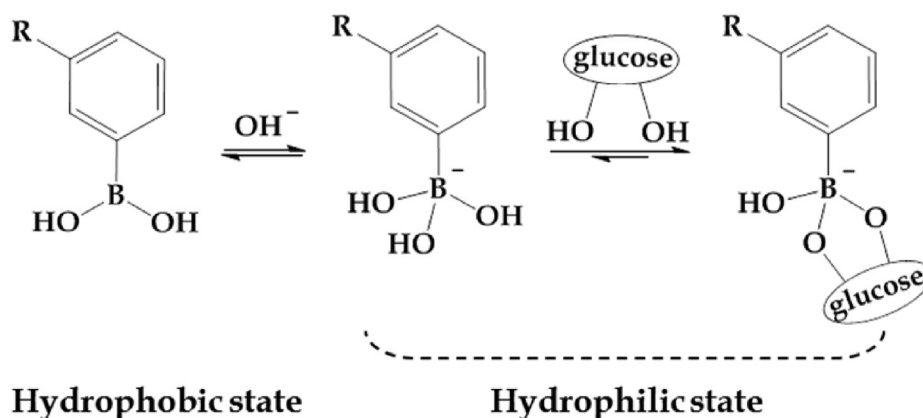Table 1 listed electrospun glucose sensors with their respective

Fig. 21. Illustrational scheme of reversible binding of glucose to PBA [164].

performances in terms of sensitivity, linear range and detection limit. Both the advantages and disadvantages of each sensor are listed in Table 2.

The best performing electrospun-based glucose sensors are narrowed down to ZnO–CuO HNCs, Ag/CuO NFs-ITO, and MnCo₂O₄. The

Table 1
Quantitative comparison of the described sensors.

Electrospun Nano material	Sensitivity ($\mu AmM^{-1} Cm^{-2}$)	Linear range (mM)	Detection limit (μM)	Ref.
Cellulose/β-CD	5.08	0–1	93.5	[100]
GOx/ NCNSs@CNFs/ GCE	13.5	0.012–1	2	[103]
PVA/BTCA/β-CD/ GOx/AuNPs NF	47.2	0.1–0.5	0.01	[105]
PEDOT NFs-GOx	6.4	0–5	260	[108]
PEDOT-NFs/GOx	74.22	0.01–1.7	2.9	[112]
GOx/Au-NPs/PANI	N/A	0.1–150	N/A	[114]
PVA/PEI/GOx	N/A	0.01–0.2	0.9	[115]
AuNPs – GO	–	0.001–0.1	0.018	[116]
Ag/CuO NFs–ITO electrode	1347	0.02–0.5	51.5 nM	[118]
FeCo@PCNSs	1766.5	0.005–1.7	0.1	[121]
L-Cys/GOx/PVA/ ZONF/GCE	70.2	0.25–19	1	[127]
ZnO–CuO hierarchical nanocomposite	3066.4	0.47–1.6	0.21	[132]
CuO NFs-ITO	873	0.0002–1.3	0.04	[73]
CuO-NFs-GCE	431.3	0.006–2.5	0.8	[135]
CuO/PCL@PPy		0.002–6	0.8	[136]
CuO/N-CNFs/GCE	968	0.25–2	N/A	[142]
Ni(OH)₂/CNF	–	0.005–13.05	0.1	[145]
NiCo₂O₄/ECF/GCE	1947.2	0.005–19.15	1.5	[148]
TiO2 hollow nanofiber	22.5		2	[149]
MnCo₂O₄ (MCFs)	679.5	0.05–800	0.01	[155]
RhCl₃/PVP	11.46	–	3.1	[156]
IrO₂ NFs-Nafion/ GCE	22.22		2.9	[157]
TiC–C nanofiber	628.2	0.013–10.5	3.7	[160]
GOD/EOF	N/A	3^{-7} - 0.13	0.0001	[162]
Au/PPyNFs	1.003	0–2	200–13000	[166]
Pt–Au nanocorals	24.6	3.2 μM - 7.2	3.2	[167]
Ni/CoO	–	0.25 μM–600 μM	0.03	[168]
NiCFP	420.4	0.002–2.5	1	[169]
Ag/CuO NFs-ITO	1347	0.0005–0.5	0.0517	[170]
NiO–Ag NFs/GCE	32.91	0–1.94	1.28	[171]
NiCo₂O₄/C	1827.5	50-400, 20–200 μM	1.2	[172]
CuFe₂O₄/Ni foam	1239	20 μM–5 mM	0.22	[173]

sensitivity of ZnO–CuO HNCs (3066.4 $\mu Acm^{-2}mM^{-1}$) is extraordinarily high compared to the other sensors listed in Table 1. The superior performance of this non-enzymatic glucose sensor is due to its special hierarchical heterojunction formation in the composite and the well-constructed 3D porous structure [132]. The thickness of the HNCs played a vital role in the amperometric sensing performance of this sensor. The interaction between ZnO–CuO in the composite structures compliments the acceleration of electron transport. The sensor was reported to show shorter response times and higher response, with the CuO NPs, implanted into mixed ZnO/CuO NWS. The hierarchical nanostructure possesses a suitable contacted area of n-type ZnO and p-type CuO (especially in NWs trunk), as appropriate p-n junctions are formed and can create strong interaction, favoring electron transmission [132]. The large surface area in the nanofibers and their 3D porous stacking modes provide a large interface and unhindered diffusion of glucose molecules during kinetic mass transfer in the electrochemical process. The 3D electrode is manipulatable in the microstructure (such as its thickness), providing better control of high-sensing performances to target molecules. It is favorable for practical applications due to its good selectivity against other interfering materials, good reproducibility, high surface area, and HNCs structure, which provide maximum numbers of active free paths of glucose molecules and facilitate faster ET (electronic kinetics) [132]. The report also shows an accurate measurement of blood serum samples relatively close to the glucose meter. This makes ZnO–CuO HNs the best glucose biosensor and is very promising for the practical application for fast diabetes mellitus detection.

Ag/CuO nanofibers-ITO, non-enzymatic glucose sensor with high sensitivity, fast response, excellent stability, and good reproducibility is comparable to ZnO–CuO HNCs sensor [170]. The report shows that the sensor can recover 96.65–100% of glucose from the human blood serum samples. However, its disadvantages lie in the low sensitivity (1347 $\mu Acm^{-2}mM^{-}1$) and the tedious fabrication procedure. Besides, MnCo₂O₄ is a complete package, where the sensor is good in all aspects. It may be the best sensor selected here due to the mediocre sensitivity (679.5 $\mu Acm^{-2}mM^{-1}$), hence it falls behind ZnO–CuO HNCs. Nevertheless, these are the top-picked sensors that are very promising for future advancement in developing glucose biosensors.

6. Conclusions and recommendations

A compilation of the various electrospinning-based fabrication methodology and electrospun materials with the focus on reliable reports in the recent progress has been presented in this review. A great focus on electrospinning technology for the fabrication of these ultra-sensitive and low-cost electrochemical and colorimetric biosensors was implemented. The effects of different fabrication process parameters and material on the sensitivity, selectivity, and LOD of the biosensors have been carefully compared and discussed in detail. An in-depth analysis has shown us a

Table 2
Qualitative comparison of the mentioned sensors.

Sensors	Advantages	Disadvantages
Au/PPyNFs	Fast, easy synthesis, anti-interfering sensors	Weak sensitivity
GOx/Au-NPs/PANI	Retain catalytic activity of glucose oxidase	Extremely sensitive to pH
PEDOT F-GOx	Retain catalytic activity of glucose oxidase at low potential compared to conducting film counterparts	Increase in potential causes out of range detection limits and non-retainable sensitivity over time (30 days), weak sensitivity
PEDOT NFs-GOx	Same advantages but higher sensitivity compared to PEDOT F-GOx by 433%	Increase in potential causes out of range detection limits and non-retainable sensitivity over time (30 days), weak sensitivity
NiCFP	Low detection limit, wide linear range, stable, low cost, and straightforward preparation method	Sensitivity lower compared to other sensors
GOx/NCNSs@CNFs/GCE	High stability, low detection limit, and wide linear range	Weak sensitivity
4-ATP/PVA/PEI/GOx NFs	Preserved enzyme activity, good stability, and very low detection limit	Narrow linear range
ZnO–CuO HNCs	High sensitivity, low detection limit, good reproducibility, outstanding long-term stability, excellent selectivity	N/A
L-Cys/GOx/PVA/ZONF/GCE	Good anti-interference ability and good long-term storage (over 4 months)	Weak sensitivity compared to others
MnCo$_2$O$_4$ (MCFs)	High sensitivity and selectivity, very wide linear range, low detection limit, fast and easy synthesis method	N/A
CuO-NFs-GCE	Fast response, good stability, high sensitivity, good selectivity, long-term storage	Almost 50% weaker sensitivity compared to CuO NFs-ITO, its successor
CuO NFs-ITO	Good stability, selectivity, and fast sensing	The experiment did not include human blood serum samples
Ag/CuO NFs-ITO	High sensitivity, low detection limit, good reproducibility, stability, and selectivity	Tedious and sensitive fabrication procedure, high NaOH concentrations affect the performance of the sensor
CuO/N-CNFs/GCE	High sensitivity, good selectivity, and stability	The experiment did not include human blood serum samples and no quantitative value for detection limit
NiO–Ag NFs/GCE	High sensitivity, low detection limit, wide linear range, excellent anti-interference property, low cost, and good reproducibility	Sensitivity lower compared to other sensors
GOD/EOF	Excellent linear range, detection limit, and response time (<1s)	No quantitative sensitivity values to do a comparison
PEG-PAM	Long-term *in vivo* CGM (140 days)	Still under-developed for any practical usage
Optode based	*In vivo* CGM	Unstable to retain at the implantation over one month Weak sensitivity
NiCo$_2$O$_4$/ECF/GCE	Wide linear range, low detection limit, facile production	
CuO/PCL@PPy/ITO		
RhCl$_3$/PVP	Good electrocatalytic activity towards glucose in human serum, reasonable selectivity	–
IrO$_2$ NFs-Nafion/GCE	Good selectivity	–
HNF–TiO2	Without impurities	–
Ni(OH)$_2$/CNF	Wide linear range, high stability, and detection repeatability.	–

mixture of both electrospun nanofibers and incorporated functional nanomaterials such as carbon-based nanomaterials, noble metal nanoparticles, metal oxide nanoparticles, and luminescent transition-metal complexes are an effective platform for the development of glucose biosensors. The versatility and simplicity of electrospun nanofibers provide a promising advancement in glucose sensor research.

Nonetheless, leaching effect of functional nanomaterials, biocompatibility, and toxicity remain the major challenges in developing glucose sensors. The toxicity of nanomaterials such as metal and metal oxide nanoparticles is yet to be fully explored. They may cause environmental pollution and serious carcinogenic effect on the person who produces and uses these sensors. Furthermore, biocompatibility and the leaching effect of nanomaterials could affect the durability and stability of electrospun glucose sensors. Though the simplicity of electrospinning technology is considered a major advantage in fabricating sensors, attempt to produce electrospun nanofiber sensor in large quantity for commercial should be explored in view of the increasing diabetic population.

Declaration of competing interest

None.

Nomenclature

Abbreviations

DET	Direct electron transfer
ISF	Interstitial fluid
CNFs	Carbon nanofibers
NFs	Nanofibers
ECFs	Electrospun carbon nanofibers
EOF	Electrospun optical fibrous membrane
NCs	Nanocorals
CNTs	Carbon nanotubes
GO	Graphene oxide
NCNSs	Nitrogen-doped carbon nanospheres
AuNPs	Gold nanoparticles
AgNPs	Ag nanoparticles
ZnO	Zinc oxide
CuO	Copper oxide
NiO	Nickel oxide
HNCs	Hierarchical nanocomposites
GCE	Glassy carbon electrode
ITO	Indium tin oxide
FTO	Fluorine doped tin oxide
SEM	Scanning electron microscopy
IEP	Isoelectric point
GOx/GOD	Glucose oxidase
CA	Cellulose acetate
BA	Boronic acid
β – CD	β – Cyclodextrin
PVA	poly(vinyl alcohol)
PVP	poly(vinyl pyrrolidine)
PEI	polyethyleneimine
PAN	polyacrylonitrile
PPy	polypyrrole
PANI	polyaniline
PEDOT	poly(3,4-ethylene dioxythiophene)
PLLA	poly(L-lactide)
PCL	Polycaprolactone

PPy	Polypyrrole
UA	Uric acid
DA	Dopamine
AA	Ascorbic acid
Trp	Tryptophan
Gly	Glycine
HRP	Horseradish peroxidase
PS	Polystyrene
PCL	Polycaprolactone
TDMAC	Tridodecylmethylammonium chloride

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