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REVIEW

Paper-Based Electrochemical Biosensors for Point-of-Care Testing of Neurotransmitters

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Abstract

Neurotransmitters are important biological molecules related to several nervous system diseases (NSDs). Point-of-care testing (POCT) of neurotransmitters is of great importance in preclinical research and early diagnosis of NSDs. Among various POCT platforms, paper-based electrochemical biosensors have achieved great advances in detection of neurotransmitters, thus taking a significant role in POCT of neurotransmitters nowadays. This review gives an overview of the recent advances of paper-based electrochemical biosensors for POCT of neurotransmitters. We first introduce the types of neurotransmitters and biological sample sources mainly used for neurotransmitter detection. Second, we review the components and the traditional fabrication technologies for paper-based electrochemical POCT biosensors, and then the functional modification methods of biosensor surfaces and three-dimensional fabrication methods for further enhancement of their detection performance. Then, we list examples of paper-based electrochemical biosensors used for detecting different neurotransmitters in biological samples. Last, we give a conclusion and promising development direction of paper-based electrochemical biosensors for neurotransmitters detection. The purpose of this review is to introduce the paper-based electrochemical biosensors as an emerging technology for POCT of neurotransmitters, offering a reference for readers and researchers for early diagnosis of NSDs using POCT technologies.

Keywords Point-of-care testing (POCT) · Nervous system diseases (NSDs) · Neurotransmitters · Paper-based electrochemical biosensors

1 Introduction

Nervous system diseases (NSDs) are one of the major public health issues in the twenty-first century [1]. Reliable early diagnosis of NSDs is of great significance for better treatment of patients and decrease of their medical expenses [2]. Neurotransmitter, e.g., dopamine (DA), acetylcholine (ACh), and epinephrine (EPI), is a kind of chemical messenger released from one neuron to another to complete cell-to-cell communication [3]. Abnormal levels of neurotransmitters during transmission and regulation are associated with several NSDs [4]. For instance, concentration of DA dropping about 25% can herald a prevalent neurodegenerative disorder, e.g., Parkinson's disease [5, 6]. Levels of ACh can drop up to 90% in patients with Alzheimer's disease which is the most common form of dementia among elderly [7, 8]. Thus, sensitively and selectively detection of neurotransmitters is necessary for the early diagnosis of NSDs [9]. However, traditional lab-based techniques to test neurotransmitters levels in vitro are commonly based on chromatograph analysis (e.g., high efficiency liquid chromatography, gas chromatography) and mass spectrometry [10], which are expensive, cumbersome, time consuming and inconvenient for preclinical diagnosis of NSDs for patients without guidance of professionals [11]. Also, conventional analysis of neurotransmitters mainly proceeds in cerebrospinal fluid since nearly all the neurotransmitters can be found in it with higher concentration than that in other body fluids [12, 13]. But cerebrospinal fluid is not an ideal human sample sources for early diagnosis of NSDs at home and source-limited

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settings since the sampling process of cerebrospinal fluid is invasive and complex [14]. Other biological fluids, such as blood, urine, sweat, saliva and tear, are more accessible and non/less-invasive than cerebrospinal fluid for clinical diagnosis and measurements [15]. To address the shortcomings of the traditional detection methods of neurotransmitters and diagnosis of NSDs, point-of-care testing (POCT) technique has been developed as an emerging technology with holding the ability of diagnosis of patients' diseases rapidly and accurately at/near the site of patients [16]. Development of user-friendly POCT platforms to rapidly evaluate the neurotransmitter levels in accessible biological fluids of patients at early stage of NSDs can remind them to seek for further diagnosis and treatment in hospital [11].

Paper with characters of low-cost, portable, disposable and compatible with biological samples has been widely used as the platform of POCT [17, 18]. Numerous paper-based POCT platforms based on different detection methods have been developed for detection of neurotransmitters [19–24]. Among various paper-based POCT platforms, paper-based electrochemical platforms have attracted special attention owning to their quantitative capability, high specificity and fast response time [25–27], thus playing a significant role in POCT of neurotransmitters nowadays. And with the recent advances in paper material, nanotechnology and instrumental miniaturization, we have also witnessed the tremendous progress of paper-based electrochemical POCT biosensors for detection of neurotransmitters [28]. For example, different kinds of papers (e.g., cellulose paper [29], conductive papers [30]) and conductive inks (e.g., carbon nanotube ink [28], graphene ink [31]) have been employed to prepare electrodes in fabrication of paper-based electrochemical biosensors for detection of neurotransmitters [31, 32]. In addition, nanomaterials with catalytic activities (e.g., Au nanoparticles, ZnO nanoflowers) have been modified on paper-based electrochemical biosensors to enhance their detection signals of neurotransmitters [33, 34]. Some miniaturized, portable testing platforms and readers have also been developed and integrated with paper-based electrochemical biosensors for detection of neurotransmitters [35]. Therefore, paper-based electrochemical biosensor, as a user-friendlyPOCT platform for neurotransmitters detection in non/less-invasive biological fluids, can serve the preclinical diagnosis of NSDs and the follow-up of patients with NSDs in the future.

Paper-based electrochemical POCT platforms for detection of neurotransmitters have been rapidly developed and the related studies have been reported in recent years [30, 36, 37]. Several previous reviews have summarized the electrochemical detection methods of neurotransmitters in laboratory [25] and paper-based POCT platforms used for biological testing [38, 39]. But a detailed review about the paper-based electrochemical biosensors used for POCT of neurotransmitters is still lacking. In this review, we intend to give an overview of the state-of-art works of paper-based electrochemical biosensors for POCT of neurotransmitters. First, we briefly introduce the types of neurotransmitters in Sect. 2 and the biological fluid types as sample sources for neurotransmitters detection in Sect. 3. Then, the components of the paper-based electrochemical POCT platforms and the fabrication methods are introduced in Sect. 4. In Sect. 5, we give some examples of paper-based electrochemical biosensors used for POCT of different neurotransmitters. Finally, we provide a conclusion of paper-based POCT of neurotransmitters and an outlook of the future development of paper-based electrochemical biosensors with portable accessory (e.g., smartphone-based reader) and other detection methods (e.g., electrochemiluminescence) for POCT of neurotransmitters (Fig. 1).

2 Types of Neurotransmitters

Neurotransmitters are the fundamental substances for transmission of chemical signals in nervous system, which are stored in synaptic vesicles of interneuronal synapses and secreted from presynaptic membrane to postsynaptic membrane for cell-to-cell communications [40]. The neurophysiological processes involved molecules are related to sleep, appetite, mood, body temperature, heart rate and several kinds of NSDs [41, 42]. Testing of neurotransmitters in healthy and pathological bodies can give insight into the mechanisms of cognition, memory, motion, addictions and diseases. In this section, the types of neurotransmitters and the relationship between neurotransmitters and NSDs are introduced and summarized in Table 1 and Fig. 1.

2.1 Biogenic Amine-Typed Neurotransmitters

Biogenic amine-typed neurotransmitters, mainly including DA, norepinephrine (NE), EPI and 5-hydroxytryptamine (5-HT), are the first type of neurotransmitters. Among these, DA influences locomotor function, psychotic symptoms, motivation, reward and addictive behaviors. Abnormal level of DA is related to several NSDs, such as Parkinson's disease [43], Alzheimer's disease [44], attention-deficit hyperactivity disorder [45], Huntington's disease [46] and schizophrenia [47, 48]. NE and EPI are responsible for central nervous system symptoms including mental confusion, disorientation and lethargy [49–51]. And they are involved in body's physical reaction to stress since their releases dramatically increase to mobilize brain and body for corresponding action by adrenal system when people are exposed to dangerous and stressful situations. Low level of NE can cause depression, while high level of NE is relevant to stress and thyroid hormone deficiency [52]. 5-HT, also named as serotonin, is

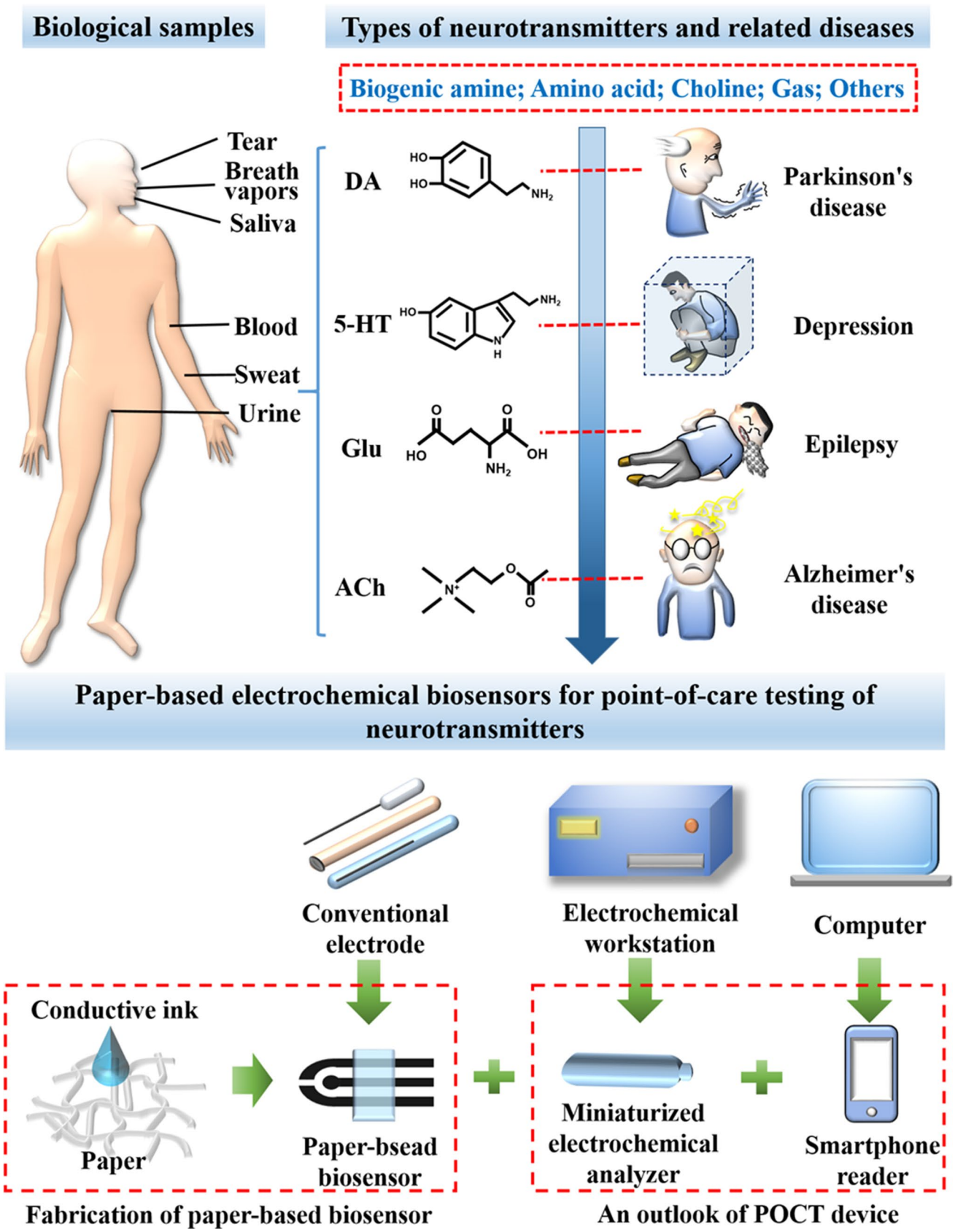

Fig. 1 Schematics of types of neurotransmitters and related NSDs and the paper-based electrochemical biosensors for POCT of neurotransmitters

Table 1 Development of paper-based electrochemical biosensors for detection of different types of neurotransmitters

Type	Neurotransmitters	Biological samples	Related diseases	Paper substrates	Conductive materials	POCT	References
Biogenic amine	Dopamine	Urine, Serum, Tear	Parkinson's disease, Alzheimer's disease, attention-deficit hyperactivity disorder, Huntington's disease and schizophrenia	Cellulose paper, graphene paper	Carbon nanotube, graphite, pencil, PEDOT:PSS	Yes	[43–48, 82, 83, 92]
	Norepinephrine	Tear	Depression postural hypotension	Cellulose paper	Carbon, boron doped diamond	Yes	[52, 88, 110, 125–127]
	5-hydroxytryptamine	Plasma, Serum	Depression and carcinoid syndrome	Cellulose paper	Carbon nanotube, Au, graphene	No	[80, 114, 128, 129]
Amino acid	Glutamate	Serum	Schizophrenia, amyotrophic lateral sclerosis, epilepsy, Parkinson's and Alzheimer's diseases	Cellulose paper,	Graphene	No	[119, 120]
Gas	Nitric oxide	/	Neurodegenerative disease	Graphene paper	Au@Pt nanoparticles	Yes	[98, 130]
	Hydrogen sulfide	/	Neurological diseases	Cellulose paper	Ag/Nafion	Yes	[99, 131–134]

a key chemical messenger in regulating mood, sleep, anxiety, appetite and sexuality. Decreases of 5-HT and NE could result in depression and further lead to persistent sadness, loss of interest and decreased energy level. Therefore, the reuptake inhibitors of 5-HT and NE are normally considered as the first-line drugs to treat depression and panic disorder aiming to increasing the 5-HT and NE levels in synaptic cleft.

2.2 Amino Acid-Typed Neurotransmitters

Amino acid-typed neurotransmitters include glutamate, aspartic acid, γ-aminobutyric acid (GABA) and glycine. Glutamate, as an excitatory neurotransmitter involved in learning and memory, acts as the most abundant chemical messenger with 9–11 μmol g^{-1} in human cortex. Certain NSDs (e.g., epilepsy) and brain injury (e.g., stroke) can cause accumulation of glutamate, which could lead to excitotoxicity, thus resulting in the final death of damaged brain cells. Aspartic acid, as an agonist for a class of glutamate receptor, is present in the central nervous system and is involved in various physiological processes, e.g., excitatory synaptic transmission, learning and memory, nervous system development, nerve cell survival and apoptosis. Abnormal aspartic acid level and activity can cause many central nervous system diseases, such as brain trauma and ischemic brain damage. GABA acts a negative feedback system to transmit "closure" signals of nerve terminals for balancing the excitation in brain [53]. The dysfunction of GABA in brain can cause epilepsy [54], schizophrenia, autism, depression, anxiety and drug addiction. Glycine as a kind of inhibitory neurotransmitters mainly exists in spinal cord and brainstem [55]. The mutation of glycine receptors in human body could cause some rare but potentially fatal NSDs, such as disorder of hyperekplexia and human startle disease [56]. In addition, with the ubiquitous distribution in nervous system and extensive involvement in NSDs, glycine and GABA have many similarities for moderating the excitability of neurons [55].

2.3 Choline-Typed Neurotransmitters

ACh as the main choline neurotransmitter in nervous system participates in physical movement, visceral movement and cardiovascular activity. Cholinergic neurotransmission throughout neocortex and hippocampus plays an essential role in arousal, learning, attention and memory processes [57] and can produce a rise in pressure through vasoconstriction [58]. The dementia caused by Alzheimer's disease is linked to ACh function [23], whose levels can drop up to

90% in the brain of the patients suffered from Alzheimer's disease. A good way to compensate low concentrations of ACh is to inhibit the acetylcholinesterase activity, which can catalyze ACh into choline and then prevent the normal breakdown of ACh [59].

2.4 Gaseous Neurotransmitters

Gaseous neurotransmitters, such as nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H_2S), function as delivering neurotransmitters [25, 60, 61]. They are essential intracellular signaling molecules, which are regulators of neuronal transmission and mediators of blood vessel relaxation [62]. NO, known as an endothelium-derived relaxing factor, takes an essential part in various biological processes [63]. Increase of NO level during epileptic activity can damage mitochondria in central neurons and lead to nerve damage [64, 65]. CO is naturally produced by human body as a neurotransmitter molecule [66]. Abnormal level of CO is linked to several diseases, including neurodegenerations, hypertension and epilepsy [66]. For example, Montecot et al. [67] found that CO can increase blood flow in brain by releasing glutamate to dilate blood vessels during seizures. Excessive increase in CO can lead to reperfusion injury and affect the recovery of nerve function. H_2S, regarded as the third gaseous neurotransmitter and with some similar biological functions of NO and CO, has a regulatory effect on the function of nervous system, especially hippocampus, and plays a significant role in the occurrence and development of various NDSs (e.g., Alzheimer's disease (AD), Parkinson's disease (PD) and febrile seizures [68–70]).

2.5 Other Kinds of Neurotransmitters

Neuropeptide, a kind of neurotransmitters for regulation of pituitary hormone secretion, includes pituitary peptide [71], tachykinin (e.g., substance P) [72, 73], cholecystokinin [74], endogenous opioid peptides [75] and orexin [76], and function as regulation of mood, stimulation of appetite and sensory state of migraine [77]. Abnormal concentration of neuropeptides is associated with schizophrenia, Huntington's disease, Alzheimer's disease and primary headache disorders [76]. Substance P, a member of the tachykinin neuropeptide family consisting of a chain of 11 amino acid residues, acts as a kind of neurotransmitter. Substance P is released from the terminals of specific sensory nerves in central nervous system (i.e., brain and spinal cord) and associated with inflammatory processes and pain [78]. Levels of substance P and its receptor in serum, plasma and tissue are associated with depression and related disorders [79].

3 Sample Sources of Biological Fluids

Analysis of neurotransmitters in cerebrospinal fluid is valuable in investigation of NSDs since nearly all the neurotransmitters can be found in it with higher concentrations than that in other body fluids [12, 13]. But cerebrospinal fluid is not the ideal candidate for preclinical diagnosis of NSDs at home and resource-limited settings because it is mainly obtained by lumbar puncture, which is invasive and could lead severe damage and serious pain [14]. In this section, we review the minimally invasive (e.g., blood) and non-invasive sample sources of biological fluids (e.g., urine, tear, saliva, sweat and breath vapors) used for POCT of neurotransmitters (Table 1, Fig. 1).

3.1 Blood

Concentrations of neurotransmitters (e.g., DA and 5-HT) in blood, including venous blood (e.g., serum, plasma) and finger-tip blood, are highly related to those amounts present in brain. Blood testing is viable for early and routine screening abnormal levels of neurotransmitters as early indicator of NSDs [80, 81]. Recently, great efforts have been made to fabricate sensitive paper-based POCT biosensors for detecting neurotransmitters in serum and plasma [35, 82, 83]. For example, Rattanarat et al. [47] reported a three-layer paper-based electrochemical device for selective determination of DA in standard serum sample. Their device showed the potential in selective quantification of DA in human serum sample with low-cost, user-friendly and portable features. Moreover, finger-tip blood, coming from nerve endings, as the most easily acquired blood sample [84], can be used as sample source of neurotransmitters for diagnosis of NSDs.

3.2 Urine

Besides blood, DA and 5-HT in urine have also been used as early indicators for diagnosis of several NSDs [85]. In comparison to blood, urine sample is simpler and more convenient to extract with a mass amount without damage through a simple dilution, filtration and direct injection, which can avoid time-consuming and tedious extraction steps [86]. The normal concentration range of DA in urine is around 0.3–2.6 μM [87]. As a non-invasive sample source, urine has been used as sample source to diagnose NSDs through detection of catecholamines (e.g., EPI, NE, DA). But on the other hand, the interferences in urine samples, such as ascorbic acid and uric acid with similar reduction potentials to those of neurotransmitters (e.g.,

DA, 5-HT), can influence the detection results of targets. To solve this, Mostafalu et al. [80] modified paper with polyaniline fiber, carbon nanotubes and rGO to achieve highly selective electrochemical detections of DA, ascorbic acid and 5-HT.

3.3 Tear

Tear is a complex biological sample secreted by lachrymal glands, containing water (98%), electrolytes, lipids, proteins, enzymes and small organic molecules (e.g., NE and DA) [88, 89]. Researchers have developed biosensors for detection of chemicals in tear [90, 91]. Concentration of DA in tear of a healthy adult is more than 1.8 mM [92]. While, a decrease concentration of DA in tear can reflect disease status [92], such as glaucoma, which is a disease partially blocks the eye innervation and reduces dopamine formation as a result. In recent years, to meet the needs of external circuit and testing equipment, researchers focused on developing flexible and minimally invasive electrochemical biosensors (e.g., contact lenses [92, 93]), which have been proven as a promising candidate for detection of neurotransmitters in tear in vitro.

3.4 Others

Besides the above three main types of biological fluids, other body fluids (e.g., saliva, sweat, breath vapors) are also biomarker-rich sample sources. Saliva has been used as biological sample to detect neurotransmitters since it contains a variety of neuropeptides (e.g., substance P, calcitonin gene) and catecholamines (e.g., NE, EPI) [94–96]. Sweat has been widely used in wearable chemical sensors with using flexible materials to detect EPI and DA [97]. Some gaseous neurotransmitters (e.g., NO, H_2S) existing in breath vapors [25, 60, 61] have also been detected using paper-based electrochemical biosensors [98, 99]. Compared with the invasive blood collection method, saliva and sweat are safer, simpler and easier to collect and thus more convenient for POCT applications.

The above six types of biological sample sources have their own advantages and disadvantages for paper-based POCT of neurotransmitters. Generally, concentrations of neurotransmitters in blood are higher than those in other biological liquids (e.g., urine, saliva) which can be obtained in an easy and non-invasive way. But the levels of neurotransmitters in the non-invasive biological liquids are comparatively low [100]. Therefore, for detection of neurotransmitters, researchers should take both the pros and cons into consideration to select the sample source of body fluids. In addition, it is essential to choose appropriate test medium, pretreatment method and test device to assess the levels of neurotransmitters for early diagnosis of NSDs [101].

4 Components and Fabrications of Paper-Based Electrochemical Biosensors

Paper-based electrochemical biosensor as an affordable, portable, disposable and low-cost platform meets the requirement of POCT [38]. Recent advances in developing paper-based electrochemical biosensors are opening routes towards POCT of neurotransmitters. The paper-based electrochemical biosensor is mainly composed of paper as substrate, electrode system as detection unit and hydrophobic channels for sample flow. In this section, we first introduce the components (including papers and inks) and the fabrication technologies of the paper-based electrochemical biosensors. Then, the modification methods for functionalization of working electrode and the three-dimensional (3D) fabrication techniques for paper-based electrochemical biosensors are given.

4.1 Papers

Paper with attractive characteristics, especially capillary force-driven sample transport based on its porous and flexible structure, has been used as POCT substrate for various analysis [37, 102]. Different kinds of papers, including bare cellulose paper [33, 36, 103, 104] and functional papers (e.g., conductive paper (graphene paper [30] and graphite paper [105])), have been applied to fabricate paper-based electrochemical biosensors for POCT of neurotransmitters [30, 36, 98]. And the physical (i.e., surface roughness, porosity) and chemical properties (i.e., surface functional groups) of paper have great influences on the ink absorbency and conductivity of electrode fabricated on paper [106]. It is thus essential to fully consider the factors of surface roughness, porosity, surface chemical properties of paper prior to selection of appropriate paper type used as substrate for fabrication of electrode. For example, cellulose papers, mainly including filter paper [33], layout paper [103], A4 paper [104] and chromatography paper [36], are the traditional paper used for fabrication of paper-based electrochemical biosensors through patterning or modifying conductive materials on them. Among these, the filter paper has larger surface area to adsorb more conductive inks for fabrication of electrode and more reagents for chemical reaction owning to its higher surface roughness and porosity compared to the layout paper and A4 paper. For instance, Guntupalli et al. [33] fabricated a filter paper-based biosensor through sequentially depositing carbon nanotubes and Au nanoparticles on a filter paper by ambient filtration method and used it to detect DA and 5-HT. In addition, since the degree of

active materials spreading over the surface of paper is controlled by modulating the interfacial energy of paper relative to the surface tension of conductive inks [106], paper modified with barrier made of hydrophobic materials or organic reagent (e.g., ethylene glycol) is more suitable for printing some electrode materials (e.g., Ag ink [107]). For instance, chromatography paper, as a kind of porous cellulose paper, is commonly used as an enzyme supporting layer for fabrication of enzyme electrode [108] and printed with hydrophobic materials to build hydrophobic channel on its surface [36]. Punjiya et al. [36] presented a 3D origami paper-based electrode using wax-printed chromatography paper as microfluidic channel integrated with portable readout instrumentation for detection of DA.

Due to the poor conductivity of original paper, which influences the ability of electron transfer, the sensitivity of the cellulose paper-based electrochemical biosensor for detection of low abundant neurotransmitters still need be improved [109]. To address this, conductive papers, such as graphene paper and graphite paper, with the properties of high conductivity, large surface area owing to their rough edges and wrinkles, and excellent electrocatalytic activity, have been used as substrate to fabricate electrochemical biosensors. Compared to the cellulose paper-based biosensors, the graphene/graphite paper-based electrochemical biosensors present low detection limit, excellent sensitivity, selectivity and stability for detection of neurotransmitters [105]. For instance, Zan et al. [98] developed a freestanding graphene paper modified with Au@Pt nanoparticles for real-time monitoring NO from cell secretion in aqueous solution and the obtained results showed a wide linear detection range from 0.4 to 673.9 μM and a low detection limit of 100 nM. An exfoliated flexible graphite paper had also been proposed as working electrode to detect DA with good detection performance with a wide linear detection range from 0.5 to 35 μM and a low detection limit of 10 nM [105].

4.2 Inks

Paper-based electrochemical biosensor is normally based on three-electrode system, i.e., working electrode, reference electrode and counter electrode. For fabrication electrodes of paper-based electrochemical biosensor, various conductive inks, including carbon ink [110], carbon nanotube ink [28], graphene ink [31], boron-doped diamond paste [110] and Ag/AgCl ink [111], have been employed through printing and writing approaches [31, 32]. Among them, Ag/AgCl ink are always used to fabricate reference electrode on paper, while others are mainly used for that of working and counter electrodes, as well as reference electrode. Carbon ink made of carbon powder or carbon paste with features of easy fabrication, low cost and potential for large-scale production is the most commonly used ink [110]. For example, Pradela-Filho et al. [104] made a paper-based electrode by writing carbon ink directly on an A4 paper by painting with a brush, and used the fabricated paper-based biosensor to detect DA. But the detection limit and sensitivity of the carbon-ink based electrode still need be enhanced since the poor conductivity and limited surface area of carbon ink, which affect the electron transfer, analyte adsorption and electrocatalytic processes on electrode surface [110]. To further enhance the electrochemical performance of the paper-based carbon electrode, inks made of other carbon nanomaterials, e.g., carbon nanotube ink [28] and graphene ink [31], have been introduced and used to pattern carbon electrodes on paper. For example, Costa et al. [28] presented a fully inkjet-printed paper-based electrochemical biosensor, which was consisted of carbon nanotubes-printed working, reference and counter electrodes, and applied to detect DA with concentrations as low as 10 μM. It can be owned to the carbon nanomaterials-based electrodes exhibit excellent conductivity, large surface area, rich surface functional group and great mechanical strength for facilitating the contact of neurotransmitters and catalytic sites, as well as the mobility of charge carriers [31, 112]. In addition, boron-doped diamond paste, with wide potential window, high resistance to fouling, low background current, robust mechanical property as well as good stability in strong alkaline and acidic media, has also been used to fabricate paper-based electrode. For instance, Nantaphol et al. [110] demonstrated the use of boron-doped diamond paste as a working electrode, carbon ink for a counter electrode and Ag/AgCl for a reference electrode by screen printing on chromatography paper and used the biosensor to detect NE and 5-HT separately.

4.3 Fabrication Technologies

A variety of technologies, including printing (e.g., screen printing [31], inkjet printing [28], wax-printing [109]), writing [82] and vacuum filtration technologies [105], have all been utilized to pattern conductive inks as electrodes and hydrophobic materials as hydrophobic channels for sample flowing on paper (Fig. 2a–c). Among them, based on the super stable performance, good reproducibility and mass production capability, screen printing is the most popular technique for fabrication of electrodes on paper [31, 103]. For example, Punjiya et al. [111] fabricated a paper-based electrochemical biosensor consisting of a carbon working electrode and an Ag/AgCl reference electrode through screen printing carbon ink and Ag/AgCl on paper, respectively. Nontawong et al. [103] presented a paper-based electrochemical biosensor for DA detection, in which the working, counter and reference electrodes were screen printed with modified graphite paste on filter paper (Fig. 2a). But the need of expensive equipment to fabricate template and the complex fabrication procedure limits the further application

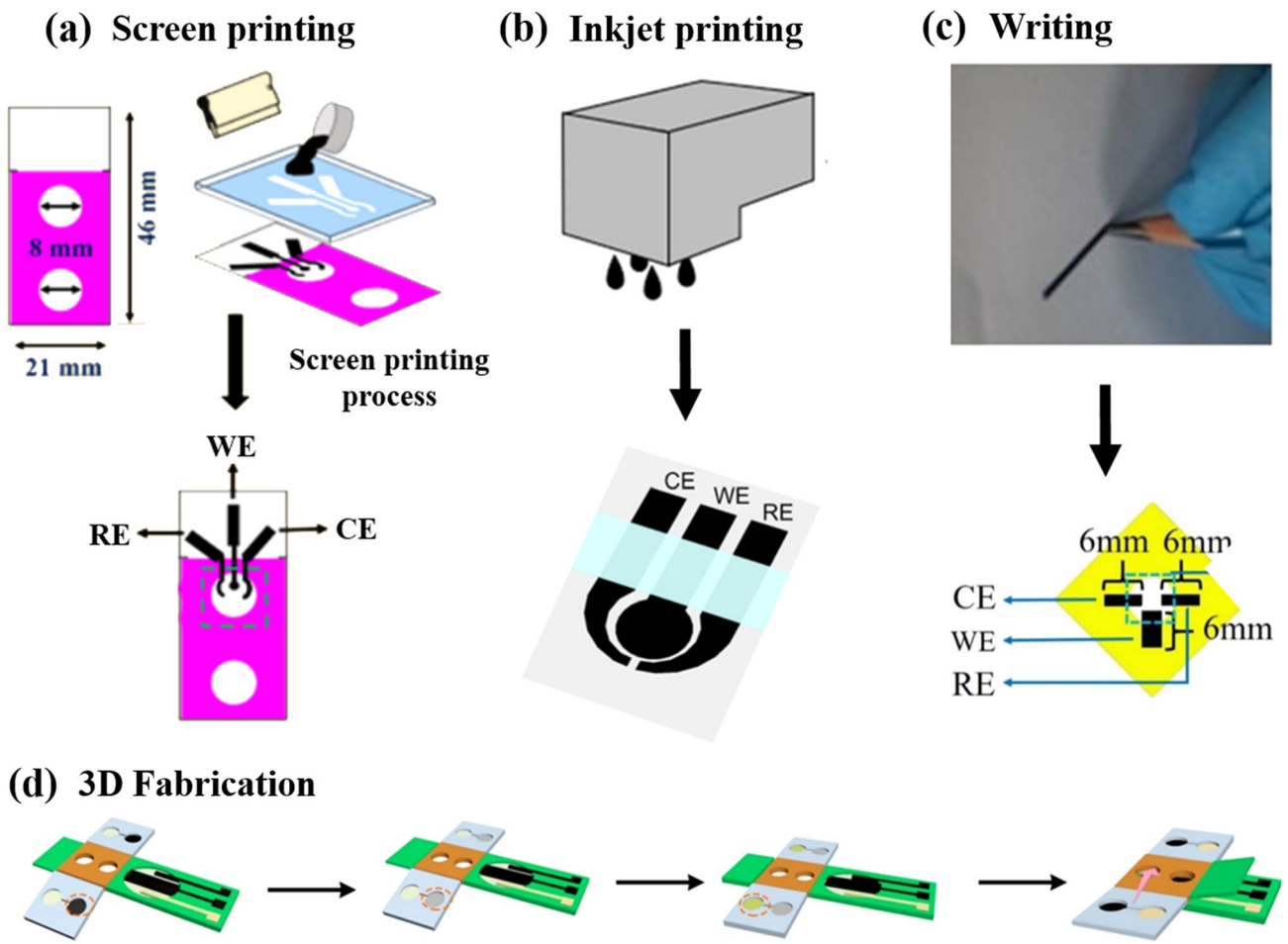

Fig. 2 Fabrication technologies of paper-based electrochemical biosensors. **a** Screen printing. Three-electrode system was screen printed on the detection zone of paper substrate using graphite paste modified with Fe_3O_4@Au-Cys/polyaniline [103]. **b** Inkjet printing. Mixing of carbon nanotube ink in deionized water, followed by inkjet printing the carbon nanotube ink on paper [28]. Adapted with permissions from [28, 103]. **c** Writing. Three-electrode system was drawn on the working zone using a commercial graphite pencil [82]. **d** 3D fabrication. The fabrication and operational procedures of the 3D paper-based biosensor conducted by a folding method [34]. Reprinted with permissions from [34, 82]. Copyrights Elsevier

of screen printing in preparation of paper-based electrodes, especially in resource-limited settings and on-site scenario [103]. To simplify the fabrication step, the inkjet printing method, which is used to simply deposit conductive inks on paper from ink cartridges, has been applied to print paper-based electrodes. For example, Costa et al. [28] printed carbon nanotube ink on paper by an inkjet printer to fabricate working, reference and counter electrodes and used the prepared biosensor to detect DA with a linear detection range of 10–100 μM (Fig. 2b). In addition, a paper-based microfluidic device combined with electrochemical biosensor, in which the patterned electrode array was made of carbon nanotube ink printed on A4 paper by an inkjet printer, has also been developed and integrated with a portable electrical control system for POCT of DA with a detection limit of 0.5 μM [35]. Furthermore, integration of screen printing and ink printing technologies together was also applied to fabricate electrode system on paper [113]. For instance, Tortorich et al. [113] fabricated paper-based electrodes through combining inkjet printing to fabricate working and counter electrodes, and screen printing to fabricate reference electrode. Moreover, in order to separate the hydrophilic reaction zones and hydrophobic channels, wax printing [109] and inkjet printing [103] have also been employed to pattern hydrophobic barriers (e.g., wax, alkyl ketene dimer) on paper for detection of hydrophilic samples on electrodes.

Recently, to meet the demands of onsite and DIY design, a pen-based writing strategy has also been proposed as an alternative approach of printing methods to fabricate electrodes on paper [82]. Different types of pens, including pencil, brush pen, fountain pen and ball pen, have all been used to fabricate paper-based electrodes [32]. For example, Li et al. [82] used a commercial pencil made of graphite particles and clay to write carbon electrode on filter paper

to detect DA in human urine and plasma. (Figure 2c). To prepare regular shaped pencil-drawn electrodes, Dossi et al. [11] used a wax printing method to pattern hydrophobic barrier on filter paper and then drew working, reference and counter electrodes at the end of hydrophobic channels by pencil, which was successfully applied in detection of DA. Besides, vacuum filtration method [105], which can form a compact, randomly arrayed, conductive layer with a maximum interpenetration of conductive ink and paper substrate, has also been utilized to fabricate paper-based electrodes. Guntupalli et al. [33] prepared paper-based porous Au films by filtrating in turn carbon nanotubes and Au nanoparticles to detect DA and 5-HT in phosphate buffer saline (PBS).

4.4 Functional Modification and 3D Fabrication Methods

The technologies introduced above mainly serve for fabrication of two-dimensional (2D) paper-based electrochemical biosensors. Due to the limited specific surface area and reactive sites, the detection sensitivity and low detection limit of the traditional 2D structured paper-based electrochemical biosensors still need to be enhanced to meet the needs of detection of neurotransmitters with ultralow concentrations. It prompts the applications of surface modification methods to functionalize electrode surfaces and the 3D fabrication methods to build paper-based electrochemical biosensors. For example, nanomaterials, e.g., carbon nanotubes, Au nanoparticles, ZnO nanoflowers, with excellent conductivity and catalytic performance have been employed to modify working electrode to increase the detection sensitivity, specificity and selectivity of neurotransmitters. For instance, Kong et al. [34] built a paper-based photoelectrochemical biosensor featured with 3D ZnO nanoflowers and polymer membranes for highly sensitive detection of glutamate. Raj et al. [31] fabricated a paper-based electrode modified with graphene and conducting polymer to simultaneously determine DA and 5-HT in urine and blood samples.

Besides functional modification of paper-based electrode surfaces, 3D fabrication technology as another emerging approach has also been employed in fabrication of paper-based biosensors. For example, researchers used 3D fabrication methods, e.g., stacking or folding paper layers, to build 3D structures of paper-based biosensors for neurotransmitters detection, which presented increased electrochemical reaction area and improved detection sensitivity of neurotransmitters compared to the traditional 2D-structrured ones [34] (Fig. 2d). In addition, the layers of 3D paper-based electrochemical biosensors can be made by patterning different functional inks and channels on paper for fluid flow [38]. Rattanarat et al. [47] reported an electrochemical paper-based biosensor containing three layers, in which the top layer was a photoresist defined hydrophilic channel on filter paper, the bottom layer was a screen-printed carbon electrode, and the middle layer was a transparent film including two holes (one for sample pre-concentration, the other for transferring the negatively charged surfactant to the bottom layer). The 3D paper-based biosensor has been used to detect DA in serum sample, which exhibited a low detection limit of 0.37 μM and a high detection reproducibility [47]. Nontawong et al. [103] fabricated a 3D paper-based electrochemical biosensor by screen printing graphite paste modified with Fe_3O_4@Au-Cys/polyaniline on filter paper and then folding underneath the hydrophobic zone on another paper made by inkjet printing. The fabricated biosensor was successfully used to DA with a linear detection range from 20 to 1000 μM and a low detection limit of 2.19 μM.

5 Applications of Paper-Based Electrochemical Biosensors for POCT of Neurotransmitters

5.1 DA Detection

In all neurotransmitters mentioned above, DA is a main target for electrochemical detection. In POCT of DA using paper-based electrochemical biosensors, cellulose papers modified with various conductive materials (e.g., carbon paste, carbon nanotube, boron-doped diamond) for specific recognition of DA have been used to detect DA in biological fluids [28, 110, 114]. For example, Costa et al. [28] printed carbon nanotubes on paper and used the prepared biosensor to electrochemically detect DA by potential step voltammetry in PBS with a linear detection range of 10–100 μM. For further detection of DA in real biological samples, Feng et al. [29] integrated a paper-based platform with electrodes made of carbon tape and treated by oxygen plasma for enhancing the interfacial adhesion to detect DA extracted from rat striatum using differential pulse voltammetry with a detection range from 10 nM to 1 μM (Fig. 3a). Furthermore, to minimize the interference effects of other coexisted electroactive compounds in real biological samples (e.g., uric acid and ascorbic acid) on detection of DA, the strategy of functional modification of working electrode surface for specific identification of DA was proposed [47]. Rattanarat et al. [47] reported a three layer-structured paper-based biosensor composed of a screen-printed carbon electrode layer modified with anionic surfactant to electrostatically interact with cationic DA. The result of square-wave voltammetry exhibited a smaller oxidation peak potential of DA compared with those of ascorbic acid and uric acid and thus could well selectively determine DA levels in serum sample without interferences from ascorbic acid and uric acid. Furthermore, to achieve a lower detection limit of neurotransmitters in human body fluids, the emerging application of

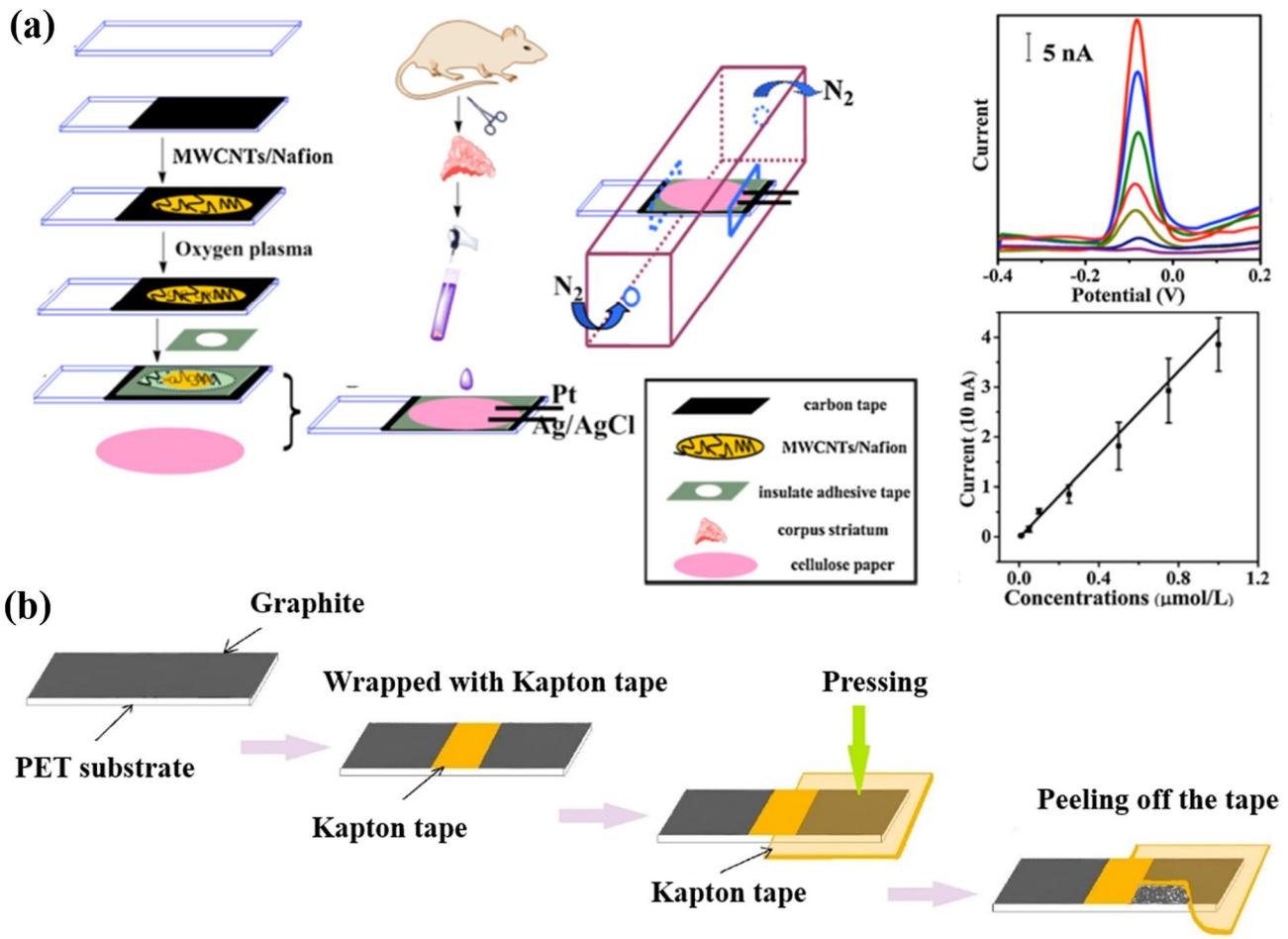

Fig. 3 Paper-based electrochemical biosensors for DA detection. **a** Preparation of paper-based electrochemical sensor based on multi-walled carbon nanotube/Nafion (left) and the application of DA detection through the method of differential pulse voltammetry (right) [29]. **b** Schematic illustration of the fabrication process of flexible electrochemical sensors for the determination of DA [105]. Reprinted with permissions from [29, 105]. Copyrights Elsevier

3D fabrication methods contributes to the development of paper-based electrochemical biosensors. For instance, a 3D paper-based electrochemical biosensor fabricated by screen-printing graphite paste on filter paper and folding hydrophobic zone on another paper was utilized to specifically recognize DA in urine using differential pulse voltammetry with the detection result without other interference and a detection limit of 2.19 μM [103].

Besides the common cellulose paper-based electrochemical biosensors, some functionally conductive papers-based biosensors have also been used to detect DA [30, 33]. For example, Zan et al. [30] used graphene paper modified with platinum nanoparticles to ultrasensitively determine DA secreted by living cells using differential pulse voltammetry with a high detection sensitivity of 2 μA μM^{-1} cm^{-2} and a low detection limit of 5 nM. Cai et al. [105] reported an exfoliated graphite paper with rough edges as working electrode to determine of DA using differential pulse voltammetry with a linear range of 0.5–3.5 μM and a detection limit of 0.01 μM (Fig. 3b). Besides using the traditional "bulky" electrochemical workstation, integration of paper-based biosensors with miniaturized electrochemical analyzers becomes more and more popular for POCT of DA. For example, Punjiya et al. [111] integrated a paper-based electrochemical biosensor made of screen-printed electrodes with a custom-designed potentiostat as the miniaturized reader and used that system to successfully detect DA via cyclic voltammetry in PBS. A smart phone-based electrochemical system was also developed and combined with a paper-based biosensor to detect DA in artificial urine [115].

5.2 5-HT Detection

5-HT as another important neurotransmitter has also been successfully detected using the paper-based electrochemical biosensors in recent years [110, 116–118].

For instance, Gomez et al. [118] reported a paper-based electrochemical biosensor made of screen-printed electrodes modified with carbon nanotubes and graphene for determination of 5-HT by differential pulse voltammetry in small sample volume (50 µL). Guntupalli et al. [33] used a paper-based electrochemical biosensor, which was made by depositing highly conductive Au films composed of Au nanoparticles and single-walled carbon nanotubes on filter paper, to detect 5-HT by cyclic voltammetry in PBS (Fig. 4a). In addition, to conquer the fouling problem of carbon electrode, which could lead to the decrease of detection sensitivity of 5-HT, Nantaphol et al. [110] demonstrated a paper-based electrochemical biosensor with using boron-doped diamond paste as a working electrode material and utilized it to detect 5-HT using both cyclic voltammetry and differential pulse voltammetry in PBS. The result showed a linear detection range from 0.5 to 7.5 µM with a sensitivity of 0.069 µA μM^{-1} and a detection limit of 0.5 µM (Fig. 4b). Even the detection of 5-HT has been realized using the paper-based electrochemical platforms introduced above, due to the irreversible consumption of 5-HT in electrochemical reaction and low

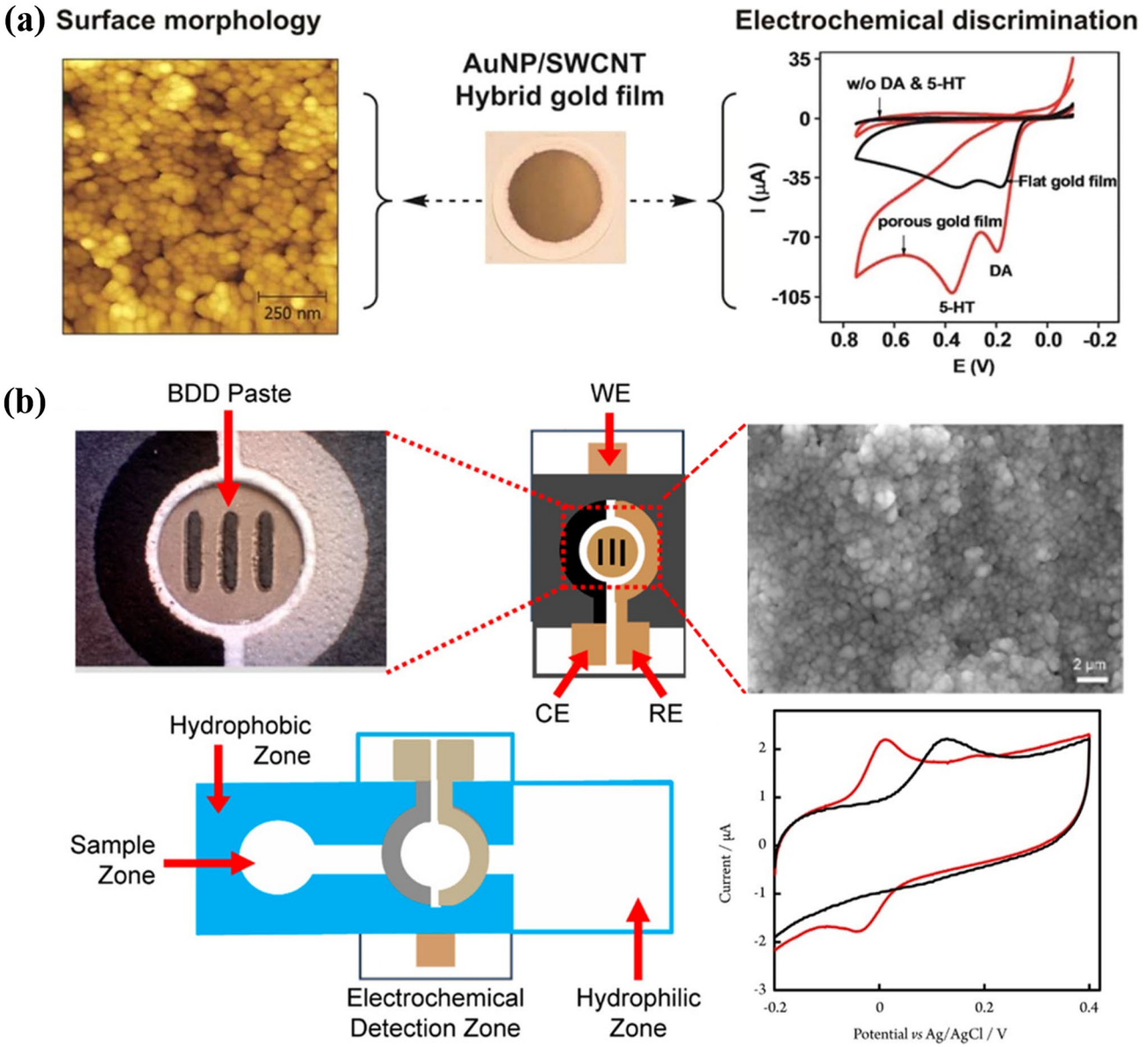

Fig. 4 Paper-based electrochemical biosensors for detection of 5-HT. **a** Morphology of highly conductive, paper-based porous Au films and simultaneous detection of DA and 5-HT [33]. **b** Morphology of paper-based electrochemical biosensors modified with boron-doped diamond and the cyclic voltammograms of the paper-based electrochemical biosensors for separate detection of NE (red line) and 5-HT (black line) [110]. Reprinted with permissions from [33, 110]. Copyrights (2015) and (2017) American Chemical Society

level of 5-HT in real body liquids, it is still a challenge to detect 5-HT in clinical samples.

5.3 Glutamine Detection

Glutamate as the most typical amino acid neurotransmitter has also been detected by paper-based electrochemical biosensors [34, 119, 120]. Generally, for electrochemical detection of glutamate, glutamate oxidase was fixed on the working electrode surface for specific recognition of glutamate [120]. For example, platinized carbon and multi-walled carbon nanotubes were printed on paper and linked with glutamate oxidase as paper-based enzyme electrodes and the prepared biosensor was used to detect glutamate in brain extracellular fluid with lower concentrations [121]. But due to the usage of enzyme, the life of electrode is limited and its stability is affected since the requirement of storage condition to keep enzymatic active. Thus, some non-enzymatic glutamate biosensors modified with nanomaterials as an alternative catalyst of glutamate oxidase have been built. For instance, Ge et al. [119] developed an Au nanoparticle-modified paper working electrode through growth of Au nanoparticle layer on the surface of cellulose fiber and used the fabricated biosensor to detect glutamate by differential pulse voltammetry, which presented a linear detection range from 1.2 to 125.0 nM and a low detection limit of 0.2 nM. Furthermore, Kong et al. [34] presented a highly sensitive paper-based photoelectrochemical sensor based on reversible photo-oxidation products and 3D ZnO nanoflowers for highly sensitive detection of glutamate in PBS with detection limit as low as 9.6 pM, which also shows potential for determination of other amino acid neurotransmitters (Fig. 5a–d).

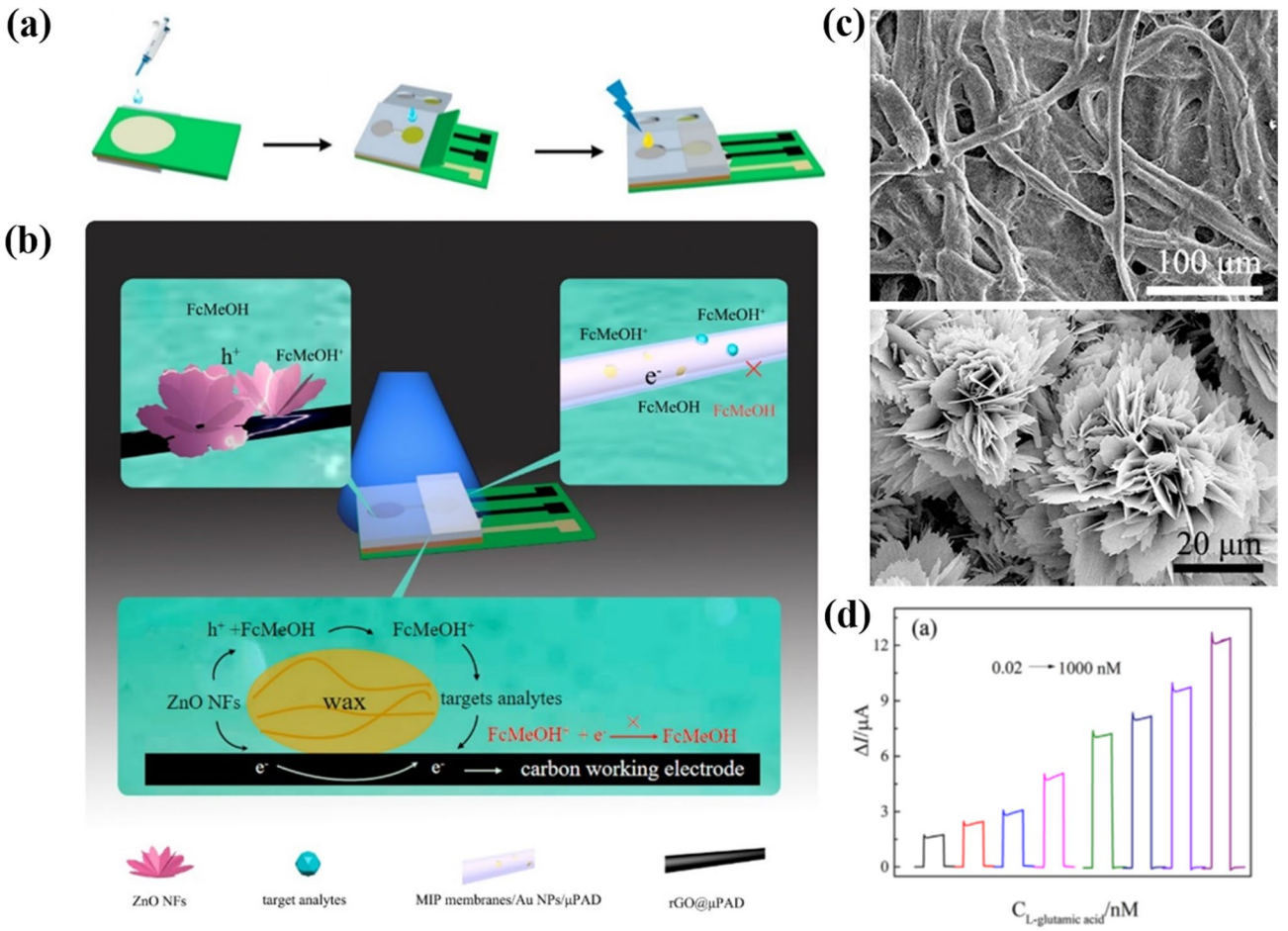

Fig. 5 Paper-based electrochemical biosensors for detection of glutamine [34]. **a** Operational process of the integrated paper-based electroanalytical devices for glutamine detection. **b** Schematic diagram of glutamine detection with photoelectrochemical analytical platform and photocurrent responses based on paper. **c** SEM images of rGO/microfluidic paper-based analytical device (top), and ZnO nanoflowers (bottom). **d** Relationship between glutamine concentration and photocurrent response ranges from 0.02 to 1000 nM. Reprinted with permission from [34]. Copyright Elsevier

5.4 Gaseous Neurotransmitters Detection

Recently, paper-based electrochemical biosensors have also been rapidly developed and used for POCT of gaseous neurotransmitters (e.g., NO, H_2S) [122]. To lower the cost and enhance the detection sensitivity, a novel kind of flexible electrochemical biosensor based on graphene paper modified with closely-packed Au@Pt core–shell nanoparticles was developed and used to real-time monitor cell secretion of NO [98] (Fig. 6a). The flexible graphene paper-based biosensor responded linearly to NO concentration up to 673.9 μM with a low detection limit of 100 nM and a high detection sensitivity of 3.653 μA μM^{-1} cm^{-2} from the obtained amperometric *i–t* curves. To determine more gaseous neurotransmitter by low-cost and miniaturized POCT system, Nechaeva et al. [99] developed a paper-based electrochemical biosensor including hydrophobic zones and hydrophilic channels using wax dipping method. In their work, after liquid-phase micro-extraction of H_2S from fuel oil sample, the paper-based electrochemical biosensor was used to selectively and sensitively detect H_2S using cyclic voltammetry with a good linear detection range from 2.0 to 20.0 mg kg^{-1} and a detection limit of 0.6 mg kg^{-1} (Fig. 6b). The biosensor can be further used to detect gaseous neurotransmitters from breath vapors in body fluids in the future.

5.5 Simultaneous Detections of Several Neurotransmitters

In real body fluids, different neurotransmitters coexist and can be used together to more accurately assess NSDs. In POCT field, the simultaneous detections of several neurotransmitters using paper-based electrochemical biosensors have several advantages, especially assessing one disease based on various neurotransmitters results to save time and enhance accuracy, thus receiving considerable attention. For example, Guntupalli et al. [33] fabricated a paper-based electrochemical biosensor made of highly conductive Au film on mixed cellulose ester filter paper, which had a larger electroactive surface owning to the maximum interpenetration and minimal aggregation of conductive materials and paper and used it to simultaneously detect DA and 5-HT by cyclic voltammetry in PBS (Fig. 4a). The boron-doped diamond paste electrode based on paper exhibited excellent performance to detect NE and 5-HT simultaneously by cyclic voltammetry and differential pulse voltammetry with wide concentration ranges (2.5–100 μM for NE and 0.5–7.5 μM for 5-HT) and low detection limits (2.5 μM for NE and 0.5 μM for 5-HT) [110]. Compared with the traditional polycrystalline boron-doped diamond electrodes (~ $310/each), the boron-doped diamond paste electrodes

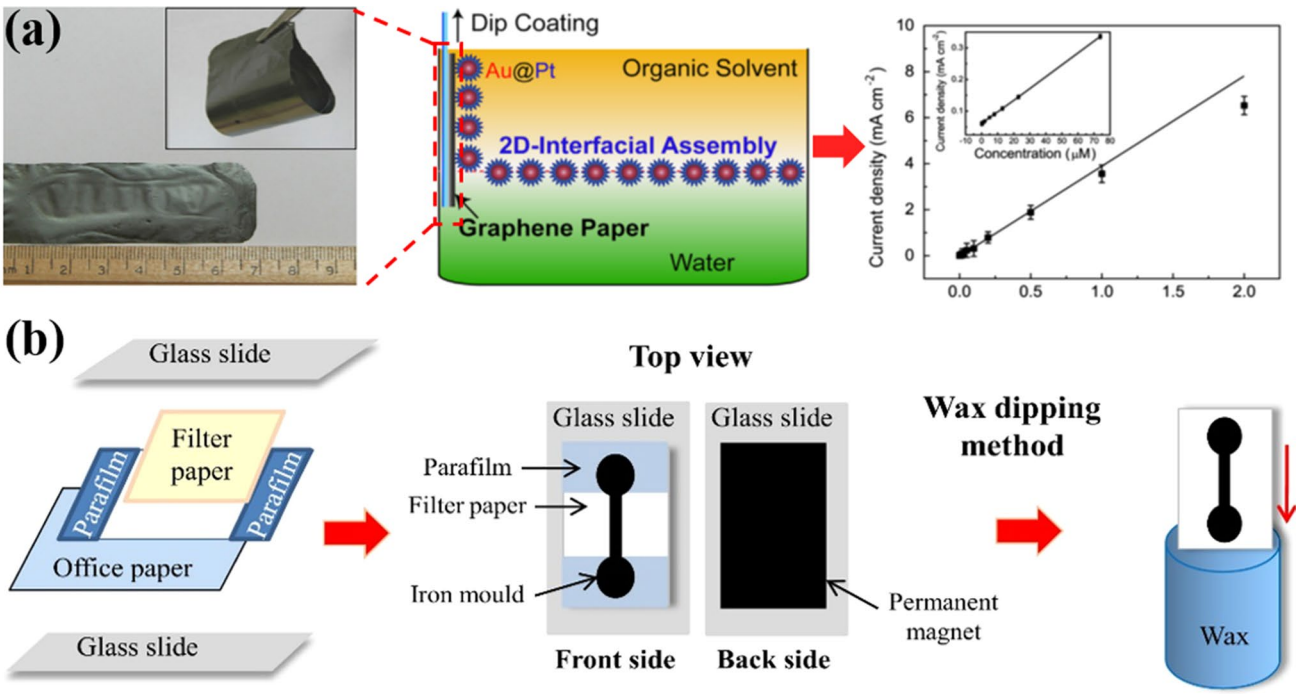

Fig. 6 Paper-based electrochemical biosensors for detection of gas neurotransmitters. **a** Morphology of rGO paper (left), schematic illustration of the fabrication of the freestanding hybrid electrode from 2D-assembly of Au@Pt nanoparticles and rGO paper (middle) and the linear relationship between peak current vs. concentration of NO (left) [98]. **b** Preparation of paper-based analytical device for determination of H_2S [99]. Adapted with permissions from [98, 99]. Copyrights Elsevier

are more cost effective (~ $0.1/each) and portable (Fig. 4b). In addition, traditional applications of paper-based electrochemical biosensors for neurotransmitters detection mainly use "big" electrochemical workstation, recent advances in the low-cost and portable paper-based biosensors, miniaturized electrochemical analyzers and user-friendly readers are opening routes towards POCT of neurotransmitters [35]. Kagie et al. [93] introduced a miniaturized and flexible film electrode on paper as a minimally invasive electrochemical biosensor for monitoring NE and DA through chronoamperometry in tears in vitro with detection limits of ~ 165 nM of NE and 530 nM of DA, respectively.

6 Conclusion and Future Perspectives

In this review, we summarized the advances in paper-based electrochemical biosensors for POCT of neurotransmitters. The types of neurotransmitters, sample sources of biological fluids, fabrication method of paper-based electrochemical biosensors are given (Fig. 1). The applications of paper-based electrochemical biosensors for POCT of several typical neurotransmitters are reviewed in detail, which includes types of neurotransmitters collected both from minimally invasive and non-invasive sources (e.g., blood, urine, tear) tested by paper-based electrochemical biosensors fabricated by various papers and inks. For more accurately assessment of NSDs, detection of two and more neurotransmitters coexisted in the same body fluids using paper-based electrochemical biosensors has attracted more attention in recent years.

Paper-based electrochemical biosensors have achieved great advances in the past few years [123]. Although the paper-based electrochemical biosensors hold obvious merits and superior prospects in POCT of neurotransmitters, there are still some challenges for their further applications. First, the application of paper-based electrodes for neurotransmitters detection is mainly performed in the laboratory at present. To realize their future practical applications, more efforts are needed to improve their identification capability of neurotransmitters and repeatability, and realize the mass production of paper-based electrochemical biosensors. Second, a lack of portably and universally electrochemical device is a hurdle for detection of neurotransmitters in real point-of-care circumstance. Last but not least, the different neurotransmitters related same disease require for multiplexed detection of neurotransmitters, which is still a challenge for the present paper-based electrochemical biosensors [102]. Therefore, the exploration of low-cost, highly sensitive and high throughput paper-based electrochemical biosensors for POCT of neurotransmitters is a nascent field of research.

Fortunately, simplicity, low-cost and easy disposal paper-based electrochemical detections of neurotransmitters in biological samples have been demonstrated as promise options in these areas. Some tunable micro/nanostructured papers (e.g., graphene-cellulose paper, Ag nanowires paper) have also been applied in optimized design and fabrication of paper-based electrodes [106]. Unlike most previously reported bulk instruments of neurotransmitters detection, portable and miniaturized POCT platforms integrated with paper-based electrochemical biosensor enable low-cost, disposable, quantitative, high specific and fast detection of neurotransmitters. More electroactive neurotransmitters can be detected through POCT platform integrating miniaturized electrochemical device with smartphone reader (e.g., smartphone and laptop) in the future [124] (Fig. 1). High-throughput and multiplex detection of various neurotransmitters can be further used for assessing NSDs accurately. In addition, the combination of electrochemical method with other paper-based detection methods, such as colorimetric, fluorescent and electrochemiluminescence, has potential to improve the accuracy and credibility of the detection results of neurotransmitters.

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