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Epilepsy Biomarker Detection: Advances, Challenges, and Future Directions

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Abstract: Epilepsy is a common chronic nervous system disease affecting about 50 million people worldwide. Traditional diagnostic methods such as video EEG have limitations such as time consuming, high cost and lack of specificity. In recent years, biomarkers such as purine, miRNA and malondialdehyde (MDA) have provided new possibilities for the early diagnosis and disease monitoring of epilepsy, and biosensor technology has shown great potential for its fast, portable and low invasive. This paper aims to review biosensor-based epilepsy biomarker detection technologies, focusing on the mechanism, detection characteristics, application scenarios and limitations of SMARTChip (detecting purine in blood), qPCR (detecting miRNA expression) and MDP probe (detecting MDA level). By comparing their technical performance, sample requirements, and clinical applicability, the study found that SMARTChip is suitable for emergency screening, qPCR performs well in laboratory diagnosis, and MDP probes provide a real-time imaging tool for oxidative stress studies. However, these technologies still need to address the process of clinical translation. This paper highlights the importance of biosensors in improving the diagnosis and management of epilepsy, and suggests that future studies should enhance marker specificity, optimize device portability, and develop multi-modal monitoring devices to promote their widespread use in epilepsy management.

Keywords: epilepsy biomarker, detection, SMARTChip, qPCR, MDP probe

1. Introduction

Epilepsy is a complex chronic neurological disorder resulting from abnormal and synchronized firing of neurons in the brain, which brings a particularly significant incidence. This not only has a serious impact on patients' physical health, but can also lead to multiple psychological, social and economic burdens [1]. At present, the diagnosis of epilepsy mainly relies on techniques such as video-EEG, which can capture the electrical activity of the brain during seizures. However, video-EEG have limitations such as time consuming, high cost, strong dependence on specialized equipment and personnel, and it cannot effectively distinguish seizures from non-epileptic seizures (such as psychogenic non-epileptic seizures), adding further complexity to diagnosis [2]. Therefore, the development of non-invasive, rapid and cost-effective diagnostic methods has become an important direction of epilepsy research. In recent years, biomarkers have become increasingly important in the study of epilepsy. Blood purines, microRNAs, malonaldehyde (MDA) and other biomarkers have been proved to be closely related to the pathophysiological process of epilepsy, providing new

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possibilities for the early diagnosis, disease monitoring and therapeutic effect evaluation of epilepsy [3, 4, 5].

Compared to traditional diagnostic methods, biosensor-based detection technology shows the potential to be fast, portable and less invasive. Biosensors enable rapid detection by specifically identifying target markers and converting them into quantifiable signals, requiring only traces of sample to achieve high sensitivity and real-time performance [6]. Apart from the high technical performance, the portability of biosensors holds promise for long-term monitoring of epilepsy in non-hospital settings, thereby improving patient management and reducing healthcare costs [7]. These advantages indicate that biosensor technology has broad prospects for clinical transformation in the detection of epileptic biomarkers.

This paper will first introduce the mechanism and characteristics of SMARTChip, real time quantitative polymerase chain reactions (qPCR) and MDP probes in detecting purine, miRNA and MDA levels, then make a comparative analysis from three dimensions of technical performance, sample requirements and clinical applicability. To provide guidance for the future development of epilepsy diagnosis and management, a comprehensive discussion of the clinical conversion prospects of these technologies and the gaps in current research will be conducted finally.

2. Current biosensor technologies for epilepsy biomarkers

2.1. Detection of blood purine levels by SMARTChips

2.1.1. Mechanism

Purines, as a class of important neuromodulator molecules, have become the focus of epilepsy biomarker research due to their release and metabolic properties in neuronal over-activity, such as epileptic seizures [8]. During seizures, neurons and glial cells release adenosine triphosphate (ATP) and adenosine via efflux or passive mechanisms, which might be quickly metabolized into inosine, hypoxanthine, and xanthine, which might enter the bloodstream later.

In the field of epilepsy, early studies focused on changes in purines in the cerebrospinal fluid (CSF). However, Bruno et al. [9] found that the level of purine in the blood of mice with single or chronic epilepsy was also elevated, suggesting that purine metabolites not only increase in concentration in the brain (such as CSF), but also may enter peripheral blood through the blood-brain barrier, providing a theoretical basis for their use as blood biomarkers. Therefore, technological breakthroughs in the detection of blood purine levels have provided a key impetus for progress in this field.

In recent years, with the development of electrochemical sensors and microfluidic technology, and the gradual maturity of point-of-care testing (POCT) technology, rapid and sensitive detection of purines in blood has become possible. Beamer et al.'s research team [10] focused on using the total amount of purines in the blood (including adenosine and its metabolites) as a biomarker for seizures and epilepsy, based on their significant elevation in both status epileptic (SE) and chronic epilepsy. They use SMARTChip (Sarissa Biomedical) technology to rapidly measure blood purine levels. SMARTChip is a point-of-care testing (POCT) technology based on enzymatic electrochemical detection, which consists of a gold electrode coated with a sol-gel layer of "ruthenium purple" containing multiple enzymes. These enzymes include adenosine deaminase, nucleoside phosphorylase and xanthine oxidase. The detection process is completed through a cascade of enzymatic reactions as shown in Figure 1. Hydrogen peroxide (H_2O_2), as an electrochemically active substance, is oxidized on the surface of the gold electrode to produce a current signal whose intensity is proportional to the concentration of the total purines (including adenosine, inosine, hypoxanthine and xanthine) in the blood [11]. The SMARTChip design includes two purine sensors and two null

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sensors, which lack sensitivity to purines, to correct non-specific interference signals and ensure measurement accuracy [10]. Each measurement is averaged (four readings: the difference between each purine sensor and each blank sensor) to further improve the reliability of the results.

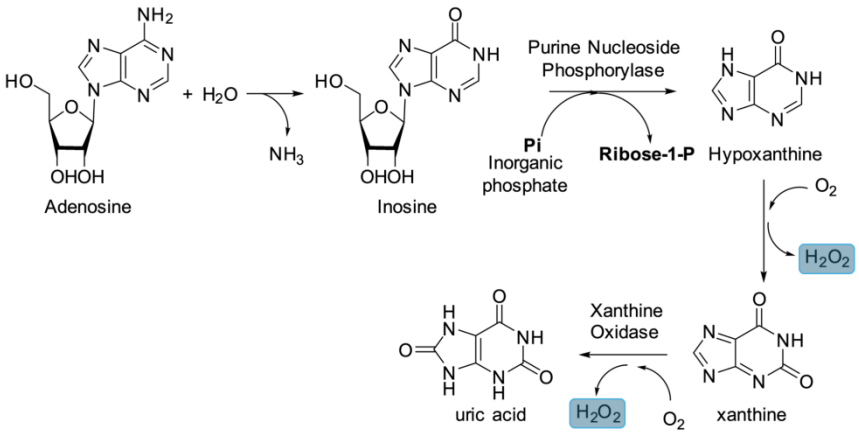

Figure 1: The detection mechanism process of SMARTChip

2.1.2. Detection features

The application of SMARTChips technology to detect blood purine levels shows significant advantages in the diagnosis of epilepsy. Compared with traditional epilepsy detection technology, the most advantage is its convenience and low sample size requirement. The technology requires only about 20 microliters of whole blood samples, and no pre-processing required, reducing the amount of blood collected and processing steps. At the same time, the SMARTChip can complete the detection of purine concentration in the blood within 3 minutes, which is of great significance for rapid diagnosis in the emergency environment.

Beamer et al.'s research team also verifies the accuracy of SMARTChip through several experiments. First, they performed linear concentration tests in phosphate buffered saline (PBS) using known concentrations of adenosine (0-20 $\mu mol \cdot L^{-1}$) and control verification by a traditional CSF testing method, "high performance" liquid chromatographic method (HPLC). The results show that the SMARTChip measurements were highly consistent with HPLC (linear regression: $r^2 = 0.997$, $y = 0.996x$), indicating its detection accuracy across a range of concentrations [10]. Besides, they added adenosine with known concentrations (0, 10, 20 $\mu mol \cdot L^{-1}$) in human serum for recovery tests, and found that the recovery was about 80%, which is consistent with the previously reported plasma recovery, further verifying its reliability in complex biological substrates.

In terms of sensitivity, Beamer et al. also tested the SMARTChip's different response to adenosine, inosine, and hypoxanthine. In the experiment, adenosine, inosine and hypoxanthine of 10 $\mu mol \cdot L^{-1}$ were respectively detected with adenosine of 10 $\mu mol \cdot L^{-1}$ as the calibration standard. It is found that SMARTChip's sensitivity to hypoxanthine is slightly higher than that of adenosine and inosine, but the overall sensitivity difference is small, indicating that the technology has a good response ability to different purine components. To target the selectivity of potential disruptors, they also tested the effects of common electrochemical disruptors on adenosine detection, such as ascorbate, paracetamol, and uric acid. The results show that these interferences alone or in combination had very little effect on SMARTChip's signal measurement of 10 $\mu mol \cdot L^{-1}$ adenosines (interference signal was less than 3%, n=9), indicating its high selectivity and anti-interference ability, ensuring the reliability of detection results. In addition, the limit of detection of SMARTChip is in the low micromolar range,

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comparable to normal blood purine levels (about 2-3 μmol·L^{-1}), which is suitable for the detection of clinical samples [12].

As for clinical diagnosis, mouse experiments have also proved that this technology is highly relevant to epilepsy pathology. Blood purine levels rapidly increased 2-3 times after epileptic status (control group: 2.32 ± 0.40 μmol·L^{-1}; After epilepsy: 8.93 ± 1.03 μmol·L^{-1}) and was significantly correlated with epilepsy severity (total EEG power and seizure burden, $p < 0.0001$) and neuronal death in hippocampal CA3 region ($p = 0.0013$) [10]. In human studies, patients with epilepsy also have significantly higher blood purine concentrations than healthy controls, suggesting that SMARTChip technology has the potential to assess epilepsy impairment and severity.

2.1.3. SMARTChips application

SMARTChip's rapid micro-sample detection features give it the potential for rapid diagnosis of epilepsy. First, its rapid diagnostic capability enables rapid detection of recent seizures in emergency or resource-limited settings to offset video EEG's time-consuming and expensive limitations, especially in situations requiring emergency intervention, such as status epileptics [10]. Second, the correlation of purine levels with epilepsy severity and brain damage makes them helpful in stratifying patients to assess severity, monitor treatment effects, and provide a basis for personalized treatment. Additionally, in drug development, SMARTChip, due to its cross-species consistency (mouse and human baseline and elevation patterns are similar), can be used as a monitoring indicator of drug efficacy in experimental models to facilitate anti-epileptic drug development. At the same time, its portability and low-cost characteristics make it promise to be popularized in non-hospital settings (such as emergency vehicles and general clinics) as a clinical diagnostic aid to improve the early recognition rate of epilepsy and quickly screen for seizures. Finally, changes in purine concentration can also play an essential role in the management of epilepsy as an assessment indicator of seizure severity and help clinicians develop more accurate treatment plans.

2.2. MicroRNAs (miRNAs) as epileptic biomarkers

2.2.1. Mechanism

With the in-depth study of the pathophysiology of epilepsy, more and more evidence show that miRNA plays an important role in the occurrence and development of epilepsy. miRNA is a single stranded RNA with a length of about 22 nucleotides, which is an important class of non-coding small RNA molecules [13]. miRNAs regulate up to 60% of protein-coding genes in the brain and are considered "meta-controllers" of gene expression. miRNAs regulate protein levels by binding to the 3' untranslated region (3'-UTR) of the target messenger RNA (mRNA), resulting in mRNA degradation or translation inhibition. The expression levels of certain miRNAs in brain tissues during epileptic seizures are significantly changed, and these changes are closely related to pathological processes such as neuronal overexcitation, neuroinflammatory response, and neuronal apoptosis [14]. The detection of these miRNAs is usually performed by initial screening of differentially expressed candidate molecules using high-throughput techniques such as microarrays and RNA sequencing, followed by real-time quantitative PCR (qPCR) to verify their expression levels to ensure the accuracy of the results [15, 16].

miRNAs are involved in the pathogenesis of epilepsy through a variety of mechanisms, including neuroinflammation, apoptosis, glial cell dysfunction, pathological neural circuit remodeling, dysregulation of nerve growth factor, and autophagy [17].

***Inflammation*:** during a seizure, brain tissue damage triggers an inflammatory response that releases several inflammatory factors (such as interleukin-1β, interferon-alpha, and tumor necrosis factor-alpha). These inflammatory factors not only destroy the blood-brain barrier and aggravate

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nervous system damage but also excite neurons and promote seizures [18]. Some miRNAs (such as miR-146a, miR-155, etc.) can reduce inflammation by regulating the expression of inflammatory factors, thus affecting the occurrence and development of epilepsy [19]. miR-146a is upregulated in epilepsy models and human temporal lobe epilepsy (TLE) to regulate inflammation and reduce IL-1 and INF-α levels by targeting the TLR4/NF-κB pathway [19]. Changes in its expression are often detected in brain tissue or plasma by qPCR. Similarly, miR-155 enhances inflammatory responses by regulating TNF-α, while inhibition of miR-27a-3p reduces inflammatory factors in epileptic rats [17].

***Apoptosis*:** when epilepsy occurs, the overexcitation of neurons can also lead to apoptosis, since miRNAs affect the survival of neurons by regulating the expression of apoptosis-related genes. Pro-apoptotic miRNAs (such as miR-34a and miR-141) are upregulated after seizure and promote neuronal death by activating caspase-3, while anti-apoptotic miR-21 and miR-184 inhibit neuronal apoptosis by targeting the mRNA of nerve growth factor-3 (NGF-3), which may play a protective role in seizures by reducing cell loss [20].

Glial cell dysfunction & deregulation of neurotrophic factors: miR-132 and miR-155 are overexpressed in glial cells and affect epilepsy by regulating pro-epileptic factors such as COX-2 and microglial cell activation [21]. miR-23a promotes glial cell proliferation and may exacerbate epilepsy [15]. miR-155 and miR-132 also affect the Brain-derived neurotrophic factor (BDNF) / tropomyosin-related kinase B (TrkB) signaling pathway and promote epilepsy, while miR-103a affects neuronal survival through regulation of BDNF expression [21, 22].

Pathological neural circuit remodeling: after a seizure, the synaptic structure of neurons will be reshaped, forming abnormal neural circuits, leading to the recurrence of epilepsy. miRNAs influence the formation and function of synapses by regulating the expression of synapse-related proteins [23]. For example, miR-132 and miR-134 can regulate synaptic plasticity by targeting p250GAP proteins, and if they are consistently upregulated, they can affect dendritic spine morphology and synaptic remodeling, resulting in neuronal overexcitation [17].

Autophagy: autophagy is a process of cellular self-degradation through lysosomes to degrade damaged or excess cellular organelles and proteins to maintain cell homeostasis [24]. The mTOR (mammalian target of rapamycin) pathway is a universal signaling mechanism involved in the regulation of cell autophagy, which is closely related to the occurrence of epilepsy [25]. miR-155 activates the mTOR signaling pathway by targeting key proteins in the mTOR pathway, such as PTEN, thus promoting autophagy [22]. miR-34a inhibits autophagy by targeting autophagy related genes such as Beclin-1, while miR-181b and miR-421 reduce autophagy by down-regulating the signaling pathway [17].

2.2.2. Detection features

Due to the stability of miRNA in biological fluids such as serum and plasma, its potential as a non-invasive biomarker for the diagnosis and prognosis of epilepsy has attracted much attention, and its detection methods mainly focus on timed quantitative real time qPCR.

qPCR is a highly sensitive technique that can quantitatively detect the expression level of a specific miRNA, usually using reverse transcription (RT) to convert miRNA into cDNA for amplification. It is suitable for small sample size analysis with fast operation and real-time monitoring of results, which can accurately detect low concentrations of miRNAs [26]. For instance, efficient quantification of mature miRNAs can be achieved by stem loop RT method combined with TaqMan probe. Furthermore, qPCR is cost-effective in certain scenarios, such as the monochrome method using SYBR Green, which reduces detection costs and is suitable for studies with limited budgets [26].

However, the disadvantage of qPCR is that the operation is complex, requiring 50-200 μL plasma samples and pre-processing steps such as RNA extraction and reverse transcription, which is time-consuming and expensive [27]. It may reduce the possibility of real-time detection in vivo and

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increase the experimental time and variability, limiting its application in bedside detection. The cost-effectiveness of multiple analysis is still limited, especially in the context of high throughput demands. In addition, miRNA expression results may vary according to the heterogeneity of epilepsy subtypes or patient populations, and standardized testing procedures are needed to improve consistency [16]. The consistency between platforms is also low, for example, the consistency of different qPCR systems for differentially expressed miRNAs is only 3%, limiting the comparability of results [26]. Therefore, qPCR is more suitable for laboratory studies or specialized hospitals where high-precision miRNA analysis is required, such as early diagnosis, disease progression monitoring, and prognosis assessment.

2.2.3. Application

miRNAs can be used as biomarkers to distinguish epileptic types (e.g., medial TLE versus focal cortical dysplasia) or to distinguish epilepsy from other neurological disorders (e.g., stroke) [17].

miRNA testing can also be used to monitor the risk of epilepsy, particularly during the epileptogenesis phase following a brain injury such as persistent state epilepsy, traumatic brain injury or stroke. Henshall [16] emphasized that changes in miRNA profiles in biological fluids after injury can predict the risk of epilepsy. For example, the expression of miR-129-2-3p in plasma is associated with seizure severity and could be used as an early marker of epilepsy occurrence and monitor disease progression [28]. Because high-throughput sequencing is suitable for finding the dynamic changes of miRNA associated with pathogenesis, qPCR can be used to monitor the expression of known markers in real time.

miRNA testing can also be used to assess the prognosis of patients with epilepsy, including surgical outcome and drug response. Ioriatti et al. [29] noted that the expression levels of miR-654-3p and miR-129-2-3p were associated with surgical outcomes in refractory TLE, and that plasma qPCR detection could predict treatment success. Besides, changes in the expression of miR-146a and miR-301a-3p are associated with drug resistance and help to adjust treatment regimen [30].

Since miRNAs can simultaneously regulate multiple pathways, targeting miRNAs provides a new strategy for the treatment of epilepsy. The effects of using anti-agomir (miRNA inhibitor) and mimic in pre-clinical studies have already been evaluated by monitoring changes in the expression of target miRNAs [16, 17]. For example, silencing miR-134 reduces seizures and provides neuroprotection by targeting Limk1 and dendritic spine density in experimental models and human TLE [23]. Neuronal death and seizures can also be reduced by anti-agomir, as miR-132 can target p250GAP and miR-34a can affect apoptosis [17].

2.3. Monitor epilepsy by MDA and MDP fluorescent probes

2.3.1. Mechanism

Oxidative stress is one of the important mechanisms in the onset and development of epilepsy and is characterized by an imbalance between reactive oxygen species (ROS) and the antioxidant defense system. As the product of lipid peroxidation, malonaldehyde (MDA) is a key marker of oxidative stress and has important significance in epilepsy [31]. During seizures, abnormal neuronal activity causes mitochondria to produce excess ROS, triggering lipid peroxidation, which increases MDA levels. A meta-analysis by Sun et al. [5] further confirmed that MDA levels in red blood cells, serum, and plasma were significantly higher in patients with epilepsy (PWE) than in healthy controls, suggesting that MDA may be a reliable indicator for assessing oxidative damage in epilepsy.

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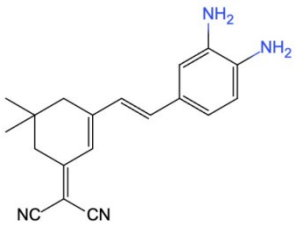

Figure 2: The structure of MDP probe

Fluorescence probe can be detected by binding with MDA through specific chemical reactions to change its fluorescence characteristics. The MDP probe developed by Duan et al.'s research team [32] is a molecule with an indene backbone (with a dicyano group) and 1, 2-diaminobenzene linked by double bonds (Figure 2). The core of the system is an extended conjugated system, in which the cyanide group in the indene part is a strong electron drawing group, while the diaminobenzene part is an electron donor group, forming a typical push-pull electron structure. This structure imparts fluorescence properties to MDP, and the 1, 2-diamino unit provides an active site for the reaction with MDA. The MDP probe uses this innovative design to transfer the fluorescence emission peak from 710 nm to 556 nm and significantly enhances the fluorescence intensity when it reacts with MDA. This change is due to the reaction of conjugated system in probe molecular structure with MDA, which leads to the redistribution of electron cloud density, and then changes the excitation and emission characteristics of fluorophore [32]. The high selectivity of the probe is due to its specific chemical reaction to MDA, which can effectively distinguish interfering substances even in complex biological environments.

2.3.2. Detection features

The MDP probe demonstrates several superior properties: First, its detection limit is as low as 0.08 μM, demonstrating high sensitivity and being able to detect small amounts of MDA [32]. Secondly, the probe showed stable reactivity in the pH range of 7-10, which is suitable for detection under physiological conditions. In addition, its high selectivity and anti-interference ability enable it to accurately identify MDA in complex biological samples. In contrast, traditional thiobarbituric acid (TBARS) detection methods, although simple, are susceptible to interference by other aldehydes and have low specificity [5]. The real-time monitoring ability of fluorescent probes is another advantage, for example, MDP probes can dynamically track MDA levels in cells and living mice, providing the possibility to study immediate oxidative stress changes in epileptic seizures.

The effectiveness of the MDP probe has been verified in cell experiments and animal models. In PC12 cells, the probe showed linear fluorescence enhancement with increasing MDA concentration (correlation coefficient R^2=0.9939), while in a mouse model of epilepsy, the probe successfully penetrated the blood-brain barrier and detected elevated MDA levels in brain tissue [32]. The meta-analysis by Sun et al. [5] also supported the validity of MDA as an indicator of oxidative stress in epilepsy, especially when it showed statistical significance in serum samples. These results indicate that fluorescence probe combined with MDA detection can provide a high-precision and reliable means of epilepsy monitoring.

2.3.3. Application

The combination of MDA and fluorescent probes has shown broad promise in epilepsy research and potential clinical applications. In basic studies, MDP probes have been used to monitor MDA

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dynamics in a mouse model of epilepsy induced by kainic acid, revealing a significant elevation of oxidative stress in brain tissue after seizure [32]. By injecting MDP probes into the tail vein, changes in MDA levels in brain tissue can be monitored in living mice, especially before and after seizures. This real-time monitoring capability helps to reveal the dynamic role of oxidative stress in the onset and progression of epilepsy, providing a new tool for studying the pathological mechanism of epilepsy.

In the clinic, MDP probes can be used to detect MDA levels in plasma or cerebrospinal fluid of patients with epilepsy as an auxiliary diagnostic tool. MDA levels may correlate with the type of epilepsy (e.g., temporal lobe epilepsy vs. generalized epilepsy) or severity, helping physicians to make more accurate classifications and diagnoses. Furthermore, MDP probes can be used for drug screening, to assess the effect of antioxidants, such as vitamin C or glutathione, in reducing seizure-related oxidative damage [31]. Clinically, this technique holds promise for real-time monitoring of epilepsy patients through non-invasive imaging, such as near-infrared fluorescence, to guide individualized treatment.

3. Discussion

Based on the three epileptic biomarker detection methods mentioned above - SMARTChip (detecting purine level in blood), qPCR (detecting miRNA expression), and MDA fluorescence probe (MDP probe) - a comparative analysis will be conducted from technical performance (sample demand, sensitivity, specificity, real-time in vivo performance) and clinical applicability.

3.1. Technical performance comparison

From a technical performance point of view, SMARTChip, qPCR, and MDP probes differ in sample demand, sensitivity, specificity, and real-time performance, so they are applicable to different scenarios.

SMARTChip, qPCR and MDP probes have significant differences in sample demand and type, so they have a great impact on the convenience of experimental operation. First, the SMARTChip requires only 20 μL of whole blood samples without preprocessing and has the smallest sample requirements and the easiest operation, making it ideal for emergency or resource-limited settings [10]. In contrast, qPCR usually requires 50-200 μL plasma samples and requires pretreatment steps such as RNA extraction and reverse transcription, which requires large sample demand and complex operation, and therefore requires high experimental conditions. However, MDP probes are mainly used for cell or tissue level detection, such as PC12 cells or mouse brain tissue. Although the sample demand is small, live or fresh tissues are needed to ensure the accuracy of fluorescence signals, and the sample type is more limited.

In terms of sensitivity, the detection limit of SMARTChip is in the low micromolar range (about 2-3 $\mu mol \cdot L^{-1}$), which is comparable to the level of purines in normal blood, so it is suitable for routine detection of clinical samples [10]. qPCR has high sensitivity for the detection of specific miRNAs, especially in low concentration samples such as plasma, but its sensitivity is limited by sample quality and primer design. In contrast, the detection limit of MDP probes is as low as 0.08 μM, showing extremely high sensitivity and being able to detect small amounts of MDA, so it is more suitable for cell and tissue level studies [32].

In the domain of specificity, SMARTChip is highly specific for purine markers such as adenosine which corrects non-specific interference through the design of two purine sensors and two blank sensors, and the test shows that its interference signal to common electrochemical interferers is less than 3%, so the specificity is high. qPCR uses TaqMan probes or specific primers to achieve highly specific detection of target miRNAs. However, its specificity depends on primer design and internal

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reference gene selection, and the internal reference stability may be affected by disease status in epileptic tissue or fluid, with potential bias. The MDP probe achieves high selectivity through specific chemical reaction with MDA and can effectively distinguish interfering substances even in complex biological environments, so it has the strongest anti-interference ability.

As for real-time in vivo performance, SMARTChip requires in vitro analysis and cannot achieve in vivo imaging. Although the detection process is fast, the real-time performance is poor. qPCR also requires in vitro analysis, including RNA extraction and reverse transcription. MDP probes support in vivo imaging and can dynamically track MDA levels in real time in PC12 cells and epileptic mouse models, providing immediate data on oxidative stress changes, thus achieving optimal real-time performance.

3.2. Clinical applicability comparison

3.2.1. Clinical applicability of SMARTChips

SMARTChip demonstrates significant benefits in terms of clinical applicability, and its portability and rapid detection capabilities make it ideal for bedside testing (POCT), which is important for rapid epilepsy screening in emergency or resource-limited Settings such as emergency vehicles or primary clinics [10]. Additionally, purine levels detected by SMARTChip are significantly associated with epilepsy severity and brain damage, and can be used to stratify patients, assess the severity of the condition, monitor treatment effects, and provide a basis for personalized treatment. Therefore, SMARTChip is best suited for rapid screening of recent seizures in emergency settings or as an adjunctive tool to assess epilepsy severity and treatment effectiveness, especially in non-hospital settings with limited resources.

However, the disadvantage of SMARTChip is its lack of disease-specific, elevated purine levels are not unique to epilepsy but are also seen in pathological states such as cerebral ischemia or traumatic brain injury [4], which limits its clinical application as an independent diagnostic tool. Thus, the future primary direction of SMARTChips development is to increase specificity by comparing purine level differences between epilepsy and non-epileptic seizures and similar pathological states (e.g., traumatic brain injury) in conjunction with other biomarkers to enhance diagnostic specificity.

Apart from the lack of specificity, it cannot achieve real-time monitoring in vivo, which limits the capture of dynamic changes. The unclear time dynamics lead to the limited detection window period. Mouse experiments showing that purine levels remain elevated for up to 4 hours after seizure, but human studies have only measured baseline levels (at least 24 hours after seizure) [10]. Thus, the temporal dynamic changes of purine levels after an epileptic seizure should be systematically studied to determine the best detection time window and explore its performance in different types of seizures to clarify the clinical application scenario.

Besides, the limited human sample size of the cast (only 26 patients, not covering different seizure types or seizure types), the need for further validation of potential disruptors (such as drug metabolites), and the unknown effects of individual differences (such as universality in different populations) also limit its broad application [10]. Under these deficiencies, a large-sample study covering patients of various ages, genders, epilepsy types, and etiologies is necessary to validate the approach's universality and reliability.

3.2.2. Clinical applicability of the detection of miRNAs

miRNAs have shown significant clinical potential in the detection of epilepsy, especially in diagnosis, prognosis assessment, and therapeutic target development. However, the clinical applicability of miRNA testing still faces several challenges. First, the inconsistencies in results across studies, such as differences in miRNA expression between different models or patient cases, limiting their

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generality. For example, in animal models and human samples, miR-146a has been repeatedly verified to be associated with inflammation, but in terms of the expression changes of other miRNAs (such as miR-199a or miR-155), the results of different models and populations vary greatly [33]. This may be due to the type of sample (brain tissue vs. plasma), type of seizure (focal vs. systemic) and the effects of anti-epileptic drugs have not been fully elucidated. Second, the specificity and sensitivity of miRNA detection need to be further optimized. Although the level of miR-19b in CSF can distinguish temporal lobe epilepsy from healthy controls [34], its specificity in other neurological diseases needs to be verified to avoid misdiagnosis. Furthermore, standardized procedures for miRNA detection have not been established, including inconsistencies in sample collection, processing, and analysis methods that may affect the reproducibility of results [17].

3.2.3. Clinical applicability of the detection of MDP probes

The greatest advantage of the MDP probe in terms of clinical applicability is that it supports real-time imaging in vivo and can dynamically monitor epileptic related oxidative stress changes. It successfully penetrated the blood-brain barrier and detected elevated MDA levels in brain tissue in a mouse model of epilepsy induced by kainic acid, providing an important tool for studying oxidative damage after seizure [32]. If further combined with near-infrared fluorescence technology, MDP probes are expected to enable real-time monitoring of epilepsy patients through non-invasive imaging to guide personalized treatment, such as adjusting antioxidant therapy regimns.

However, the shortcomings of MDP probe limit its current clinical application: the synthesis process of MDP probes is complex, which may increase the production cost and limit its large-scale application [32]; Besides, fluorescence signals may be limited by tissue depth and may be weakened by light scattering in deep brain tissue detection [35]. The detection of MDA levels may have inconsistent results due to the small sample size of sample types (such as plasma and red blood cells), suggesting that the applicability of fluorescent probes in different biological matrices needs to be further verified.

At present, MDP probes have not yet entered the clinical trial stage and are mainly limited to basic research. Therefore, MDP probes are most suitable for research scenarios, such as monitoring oxidative stress dynamics in animal models or screening novel antioxidant drugs and may play a greater role in clinical real-time monitoring in the future if costs are reduced and tissue penetration is optimized.

3.3. Future clinical potential

Technical optimization is also significant for blood purine detection, such as developing the ability to distinguish between different purine components to more accurately parse purine sources and metabolic pathways and further reduce detection costs. SMARTChips can also be integrated into existing diagnostic processes to evaluate its practical impact in clinical decision-making, explore its association with seizure-related complications (such as sudden death), and even study its potential application in other neurological diseases (such as cerebral ischemia, traumatic brain injury), thereby broadening its diagnostic value.

The use of miRNAs for epilepsy detection and integration into wearable devices is an important direction for future development, with the potential to improve the accuracy and real-time performance of epilepsy management. To achieve this, researchers can learn from the technical framework of existing wearable devices, such as the use of microfluidics to extract miRNAs from body fluids (such as sweat or saliva), combined with nano sensors for real-time detection. This approach has already shown feasibility in sweat cancer biomarker detection [36]. Specifically, a wristband device with a miniature nucleic acid amplification module (such as LAMP, an isothermal

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amplification technology) can be designed to rapidly amplify the miRNA signal, which is subsequently converted into a quantifiable electrical or fluorescent signal by electrochemical or optical sensors. In future application, machine learning algorithms can be considered to integrate into the device to analyze miRNA expression patterns and predict seizure risk. In this way, miRNA-detected wearable device not only provide a molecular level of epilepsy monitoring but may also open new avenues for personalized treatment.

In the future, MDA fluorescence probe technology may be developed too, among the possible directions are as follows: First, to improve the tissue penetration of the probe, for example, to detect MDA in deep tissues by combining near-infrared Region II (NIR-II) fluorescence technology [37]. The second is to develop multifunctional probes that simultaneously detect MDA and other markers of oxidative stress, such as 4-HNE, to provide a more comprehensive assessment of oxidative status. The third is to promote the clinical transformation of the probe, making it suitable for human trials by simplifying the synthesis process and optimizing the biocompatibility. In addition, combining artificial intelligence to analyze fluorescent signals can improve the automation and accuracy of epilepsy diagnosis. While MDP probes may play a role in real-time home monitoring if they can be integrated into wearable devices, it can monitor the oxidative stress status of epilepsy patients in real time by detecting MDA levels in body fluids such as sweat or saliva and predict seizure risk. With a better understanding of the mechanism of oxidation in epilepsy, MDA fluorescent probes are not limited to monitoring, but may also provide support for the development of novel antioxidant therapeutic strategies, such as drugs that target mitochondrial ROS production.

Advances in biosensor technology have provided new possibilities for the detection of epilepsy biomarkers, and SMARTChip, qPCR and MDP probes have made breakthroughs in this context as individuals. Nevertheless, developing from a single biomarker detection to a multi-modal might even help to optimize the clinical process. Integrating multiple biomarkers, such as purines, miRNAs, and MDA, is an important strategy to improve the specificity of epilepsy diagnosis.

Purine levels detected by SMARTChip are significantly associated with epilepsy severity and brain damage, making them suitable for rapid screening and disease assessment, to compensate for the time consuming and expensive video-EEG [10]. However, it is not specific enough and needs to be used in combination with other markers to improve diagnostic accuracy. During the detection of miRNAs (such as miR-106b-5p) in plasma, qPCR shows high diagnostic sensitivity and specificity, which can be used for early diagnosis and prognosis assessment. When integrated with purine testing, its diagnostic capabilities will be further enhanced, especially in specialized hospitals for disease progression monitoring and personalized treatment planning. Currently, MDP probes are mainly used for research, but the MDA levels detected by MDP probes reflect epileptic related oxidative stress. If clinical translation is achieved in the future, real-time monitoring can guide antioxidant therapy, such as evaluating the remission effect of antioxidants (such as vitamin C) on epilepsy, which could also consolidate SMARTChips and miRNA applications in the field of real-time epilepsy detection to achieve comprehensive monitoring in the home environment and constructing multidimensional prediction models to further optimize seizure warning and intervention strategies [32].

4. Conclusion

This paper reviews the biosensor-based biomarker detection techniques for epilepsy, and focuses on the role of purine, miRNA and malondialdehyde (MDA) in the study and clinical management of epilepsy. Studies have shown that elevated blood purine levels are closely related to epilepsy severity and brain damage and can be used to rapidly screen for recent seizures and assess the condition; Abnormal expression of miRNA provides a non-invasive tool for early diagnosis and prognosis assessment, and its high sensitivity and specificity help to distinguish epilepsy types and monitor disease progression. As a marker of oxidative stress, MDA can reflect the neuronal damage caused

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by epileptic seizure, which provides a basis for the evaluation of the effect of antioxidant therapy. By revealing the pathological mechanism of epilepsy, these biomarkers open new ways for early diagnosis, disease monitoring and personalized treatment, highlighting their important value in improving epilepsy management. However, the current research still needs to solve the problem of insufficient specificity of markers and unclear detection time window. In the future, the diagnostic accuracy should be further improved through multi-marker combined detection and larger clinical studies, and the application of these biomarkers in clinical practice of epilepsy should be promoted.

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