

Success of electrochemical biosensors for the detection of SMN1 gene products and mutations in SMA diagnosis and monitoring – A mini review

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Abstract

Biosensors have been widely used to diagnose diverse diseases, and to monitor treatment response and the course of disease in patients. Spinal muscular atrophy (SMA) is an autosomal recessive neurodegenerative disease, and the most frequent genetic cause of infant death. SMA occurs due to hotspot pathogenic mutations in the telomeric copy of the survival motor neuron 1 (SMN1) gene (homozygous deletion of SMN1 exon 7), leading to progressive death of alpha motor neurons, ultimately resulting in severe muscle weakness. Therefore, the accurate measurement of SMN1 protein levels or the determination of pathogenic SMN1 mutations in blood are needed for screening newborns for SMA, and for monitoring the progression of the disease and treatment response in patients diagnosed with SMA. This brief review aimed to demonstrate the success and future potential of biosensors in the determination of the levels of SMN1 protein levels or pathogenic SMN mutations in various clinical samples such as blood for the early diagnosis and monitoring of SMA.

Keywords: spinal muscular atrophy, survival motor neuron 1, biosensor, SMN1, diagnosis treatment response



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1. Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive neurodegenerative disease. This disease is the most frequent genetic cause of infant death: it affects around 1 per 10,000 live births [1]. It consists of a group of disorders associated with the loss of spinal motor neurons [2]. This progressive neuromuscular disorder results in muscle weakness and may even lead to death due to respiratory failure. SMA results from mutations in the telomeric copy of the SMN1 gene, but all patients have SMN2, a centromeric copy of the gene. SMA patients have homozygous loss of function of both copies of SMN1. Homozygous mutations (deletions) in SMN1 gene usually result in very low or zero SMN protein levels in the cells of SMA patients. In other words, the lack of SMN protein levels is associated with the development of SMA and related symptoms. Genetic testing for homozygous deletion in SMN1 gene in patient samples can confirm the presence of this disease in 95% of patients, independently of disease severity [3]. Molecular genetic testing is the standard test for the diagnosis of SMA, and the presence of particular pathogenic variants in the SMN1 gene can be identified with these tests. Testing parents for homozygous SMN1 gene deletions is also considered critical in the clinic [4, 5]. There is an accelerating interest in the early diagnosis of SMA in newborns, and different analytical methods have been developed and applied for the diagnosis of SMA, by the detection of SMN protein levels or the presence of pathogenic mutations in SMN1 gene, such as mass spectrometry (MS), high-performance liquid chromatography (HPLC), high-resolution melt profiling (HRM), real-time (RT) and multiplexed PCR, PCR-RFLP, multiple ligation-dependent probe amplification (MLPA), quantitative Real-Time PCR (qPCR), next generation sequencing (NGS), droplet digital PCR and various quantitative immunoassays (western blot (WB), immunoprecipitation, immunocytochemical methods, immunofluorescence, immunohistochemistry, immunostaining, electrochemiluminescence immunoassays and ELISA). However, the majority of these methods are mostly still expensive to apply, and require relatively large and expensive equipments to perform the assays; immunoassays require many reagents, involve multiple experimental steps, take time and are not quite suitable for point-of-care (POC) testing (POCT) or high throughput screening of SMA [1–5].

The most frequent mutation causing SMA is the homozygous deletion in exon 7 of the SMN1 gene, which, as described above, might be easily identified and used as a marker in sensitive diagnostic tests. In other words, in more than 95% of the patients having SMA, the disease occurs due to the presence of this single hotspot pathogenic mutation. Since SMN2 produces fewer full-length (FL) transcripts, and the levels of SMN2 copies can alter the clinical phenotype, it also becomes an important factor used in the prediction. In other words, SMN2 copy numbers provide information about the severity of the disease. SMA carrier screening methods at population levels identify carrier couples who may potentially pass this genetic disorder onto their children and enable these carriers to make informed reproductive choices or get ready for the immediate treatment of the child affected by this risk [6].

The determination of SMN protein levels present in the blood can be used to determine the response of SMA patients to treatment [7, 8]. Since insufficient SMN protein levels are observed in the blood of patients with SMA (mostly due to the homozygous mutations (deletions) in SMN1 gene), various clinical trials are being conducted to increase SMN protein levels in SMA patients, and new treatment strategies are being developed accordingly. In order to evaluate the success of such treatment approaches, it is required to determine SMN protein levels in the blood of SMA patients [8]. In other words, the determination of systemic levels of SMN protein in patients is of critical clinical importance in determining the response of patients to treatment in long-term clinical studies. It has been shown previously that SMN protein levels can also be a biomarker in determining the severity of SMA, where low plasma SMN protein concentrations are associated with the increased severity of the disease [9]. Similarly, SMN levels in the blood have been shown to be associated with SMN2 copy numbers and the severity of neuronal loss [10, 11]. In other words, SMN protein levels can be used as a biomarker in the determination of the response to treatment (therapy response) and the severity of the disease in SMA [12]. Therefore, the determination of SMN levels in the blood, in addition to the identification of the pathogenic SMN1 mutations, is ultimately of great importance for the implementation of optimal and most effective treatment, and thus increasing the overall survival rate and quality of life of patients living with SMA.

Novel diagnostic/monitoring tools such as biosensors are needed to overcome the limitations of conventional tools used for diagnosing and monitoring SMA, since these methods differing in working principle, application

areas, detection performance (such as detection limits), and cost, all require large instruments/devices and trained/professional operators, which significantly limit the broad application of these methods such as in developing countries or low-resource regions. Optical transducers have been developed to detect SMN1 gene defects, as optical nucleic acid biosensors have been previously developed and applied for the detection of single nucleotide polymorphisms (SNPs) associated with SMA [13]. Furthermore, surface plasmon resonance (SPR) has been used in order to detect SMN protein [14]. However, these optical biosensors are costly and still are not able to meet current clinical and analytical needs, highlighting the need for biosensors for the determination of SMN protein levels or pathogenic mutations in SMN gene to be used in diagnosis and monitoring of SMA, which are low cost, quick, requiring small volumes of analyte, suitable for point-of-care testing and for resource-limited settings.

In the present mini review, we covered recent studies reporting on the development of biosensors for the determination of SMN protein levels in biological fluids or pathogenic mutations in SMN gene, since these recently developed novel sensors have been comprehensively reviewed elsewhere, to our knowledge. We here aim to highlight the critical clinical importance of these sensors in the context of SMA diagnosis and monitoring, by also emphasizing the need for further research and development in this field to make these proposed sensors become widely accepted in the practical settings.

2. Biosensors developed for the determination of SMN protein levels or pathogenic mutations in SMN gene

Biosensors have found a broad range of applications in the diagnosis and monitoring of many diseases [15–18]. Different biosensing methodologies have been reported in the past to determine the circulating levels of SMN protein or to identify the presence of pathogenic mutations in SMN gene. Eissa et al. proposed an electrochemical immunosensor based on a disposable carbon nanofiber (CNF) for the simultaneous determination of three different proteins, namely, SMN1, cystic fibrosis transmembrane conductance regulator (CFTR) and DMD (Duchenne muscular dystrophy), in human whole blood samples [19]. One of the advantages of CNFs is that the surface of the CNFs is able to be functionalized with no degradation of the carbon backbone structure providing an increased surface area to immobilize biomolecules such as antibodies. The CNF electrodes in this study were functionalized through the reduction of carboxyphenyl diazonium salt electrochemically by cyclic voltammetry. Later, three antibodies against these three proteins were immobilized covalently on the working electrodes of the array sensor by using carbodiimide (EDC/NHS) chemistry. CNF-modified electrodes were then utilized as transducers for the immunosensor. Due to the particular features of CNF and the strength of the functionalized surface, the detection could be performed in a label-free manner. The authors stated that voltammetric sensors for CFTR, DMD and SMN1 have detection limits (LODs) of 0.9 pg/ml, 0.7 pg/ml and 0.74 pg/ml, respectively. Besides, this multiplexed immunosensor was also found to have high selectivity against non-specific proteins. They stated that the proposed immunosensor could be a relatively cheaper, easy, fast and high-throughput potential screening method for these three genetic disorders with very small volumes of blood analysed. The main advantages of this sensor compared to its alternatives were stated to be its fast response, lower cost and requirement of small volumes of analyte, making it suitable for point-of-care testing outside the lab. Besides, it was suggested to be suitable for the simultaneous and POCT of multiple proteins in addition to SMN1 protein in blood.

Hu et al. (2022) proposed a nucleic acid detection method based on Cas14a1, combined with asymmetric PCR to develop a method for the detection of homozygous deletion of exon 7 in SMN1 (a hot spot pathogenic mutation causing SMA) in patients with SMA [20]. CRISPR/Cas-based detection of nucleic acids offers a novel option for the genetic testing of disease-associated mutations in genetic disorders in humans. Cas14a1, a member of the Cas family of proteins, is known to exhibit great sensitivity and specificity, and it can efficiently identify between single-base differences, making it superior to some other Cas proteins such as Cas12. Cas14a1 was used for the first time in this study for detecting human genetic diseases. The analysis of genomic DNA (gDNA) which was isolated from the peripheral blood of non-SMA controls and SMA patients with this method enabled the SMA patients to be significantly distinguished from the individuals having no SMA, similar to results obtained from MLPA (multiple ligation-dependent probe amplification) and qPCR, methods used in the diagnosis of SMA. The proposed technique was reported to be promising both for the determination of SMN1 exon 7 and as an accurate detection platform for pathogenic mutations in certain genetic diseases. Compared to a similar methodology developed by Zhang et al. based on Cas12a [21],

Cas14a1-based assay developed more recently showed higher specificity and discrimination in the analysis of difference between samples from non-SMA controls and patients having SMA. With regard to the minimum measurable amount of DNA with these two SMA-Cas assays [16, 17], SMA-Cas12a used in combination with a fluorescence probe was shown to reach a detection limit of 0.1 ng/ μ L (around 52.6 aM), and the lowest concentration of DNA detected was shown to be 526 aM when recombinase polymerase amplification was used together with the SMA-Cas12a-strip assay. In the later study, the minimum concentrations of DNA detected were found to be \sim 26.3 aM with secondary PCR combined the SMA-Cas14a1 assay, and around 5.26 aM (\sim 3 copies per μ L) with asymmetric PCR used in combination with the SMA-Cas14a1 assay. This is highly important in the detection of low concentrations of DNA such as DNA isolated from dried blood spots, cell-free DNA, oral swabs and some other specimens [21]. Besides, this methodology significantly shortened the assay time due to the elimination of some experimental steps required in alternative methods (such as the absence of a PAM-sequence restriction with this Cas protein). This technique thus can be stated to hold important implications for the early diagnosis and efficient treatment of patients with SMA in the clinic [21]. Moreover, it is adaptable and scalable for the widespread detection of disease-associated mutations in some other genetic diseases in humans, in addition to SMA.

Similarly, Watterson et al. (2004) fabricated a fiber optic biosensor based on the use of probe oligonucleotides which are immobilized covalently to the surface of fused silica optical fibres, for the detection of SMA-associated SNPs in patient samples [14]. The proposed sensor is an important technique for rapid (<2 min) and quantitative SNP screening and represents a largely versatile and simplified platform technology of genetic testing, since carrying out most SNP analyses needs many hours for hybridization steps and final data analysis. This fibre-optic biosensor was shown to be highly reusable (\sim 80 cycles of application) in a clinically important range (0–4 gene copies). Oligonucleotide probes immobilized enhanced the selectivity for SNP discrimination. Sensors functionalized with these oligonucleotide probes were used in a total internal reflection fluorescence detection motif in order to determine 202 bp PCR amplicons from samples taken from patients. RT analysis was shown to be able to be performed within a range of ionic strength conditions (0.1–1.0 M) with no need for stringent rinsing to eliminate non-selectively bound materials and with no loss of selectivity, allowing a means for simple preparation of samples. Authors also stated that, by the use of the time-derivative of fluorescence intensity as an analytical factor, response linearity can be maintained, and this allows notable decreases in the time of analysis (10–100-fold), enabling the completion of measurements in less than 1 minute [14]. Combined, main advantages provided by this method are lower time of analysis (speed), and high reusability and scalability, having no requirement for stringency washing while maintaining high selectivity. Besides, samples obtained from a wide range of different preparation techniques can be possibly analyzed using this method. Therefore, this sensor format was suggested to represent a critical step for the development of biosensors for fast and quantitative screening of SNPs. However, improvements in optical design might further increase the sensitivity of detection in the future. Using this method as a basis, point-of-care analysis devices can possibly be developed in the near future.

A turn on/off biosensor for the determination of exon 7 in SMN1 gene was proposed by Chen et al. (2014) [22] using a scorpion primer which utilized the principle of fluorescence resonance energy transfer with a fluorophore, a blocker and also a quencher. The biosensor, which was successfully applied to 10 volunteers, was reported to be a highly specific method for detecting the presence of SMN1. Another SMN protein biosensor was proposed by Khoirudin et al. (2024), based on quartz crystal microbalance with dispersion (QCM-D) [23]. The QCM-D is an acoustic wave-based sensor which is able to detect small changes in terms of mass, even in the nanogram scale, and it has been used in the design of biosensors to determine proteins, DNA, and some other molecules in liquid form [24, 25]. It utilizes the piezoelectric principle with the output form of a change in frequency and energy dissipation as a sensor response against the analytes [26]. The sensor in this study was coated with polyvinyl alcohol (PVA) nanofiber and doped with SMN antibodies in order to enhance sensitivity. This sensor was found to have a good stability for 3 days, enough to detect SMN protein concentrations present in blood plasma taken from patients. The Authors reported that the presence of SMN antibodies on the biosensor surface elicits significant responses, and that the proposed biosensor has the potential to determine SMN concentrations in samples of human blood plasma. In other words, the presence of the antibodies against SMN protein in the design of sensor increased the sensor response (resulting in approximately 1.5 times higher sensor sensitivity). Authors of the study also stated that the sensor response to plasma samples obtained from SMA patients was lower compared to that of healthy (non-SMA) individuals. The results were comparable to data from Western Blot (WB), where plasma samples taken

from SMA patients had lower SMN protein levels. Besides, the authors found that the response of the developed sensor for each SMA patient was different, showing that SMA patients have differing concentrations of SMN protein in their plasma, based on the disease severity. Therefore, it was proposed in the study that this novel sensor could determine SMN concentrations quantitatively and be utilized to identify SMA severity [19].

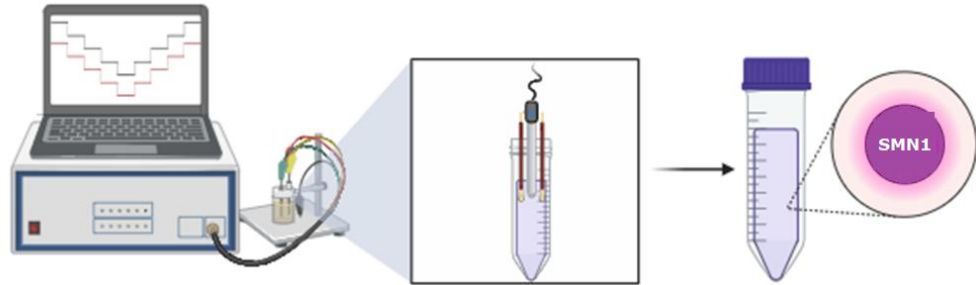


Fig. 1. An illustration summarizing the use of electrochemical biosensors for the determination of SMN1 protein levels in biological fluids including serum

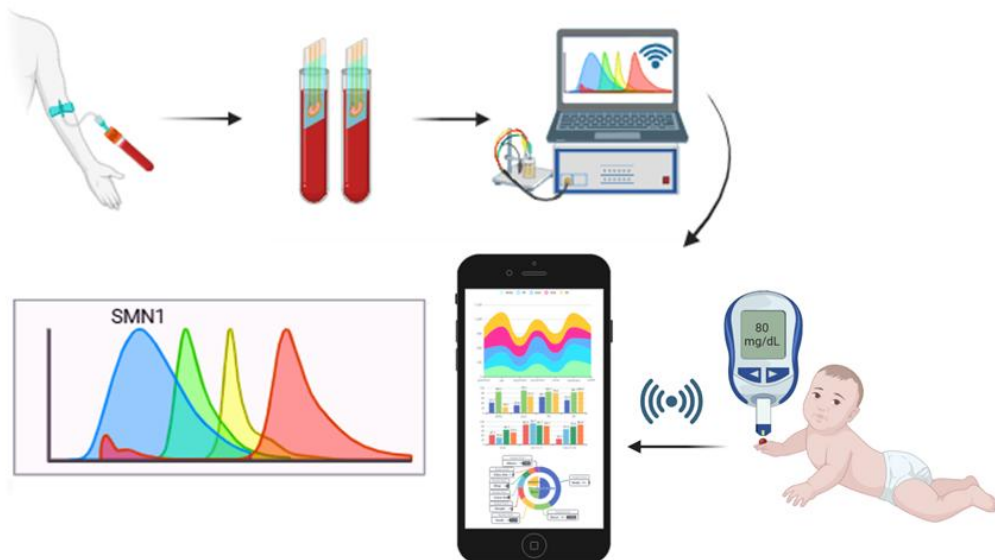


Fig. 2. An illustration summarizing the miniaturization of biosensors and android-based applications that can be used in the diagnosis of SMA.

3. Conclusion

Previous studies included in this review proved that the analysis of SMN protein levels or the identification of the presence of pathogenic mutations in SMN gene could be successfully performed using biosensors developed based on various methodologies and materials, benefiting the diagnosis, early treatment and disease monitoring of SMA (Figures 1 and 2), with particular advantages such as analysis speed, small volume requirement, high selectivity and sensitivity, low cost, suitability for POC testing, adaptability and scalability, multiplex analysis and ease of analysis. Moreover, new methodologies such as CRISPR/C as can be combined with other biosensing methods for the specific and sensitive testing of pathogenic mutations in SMA, for instance, the deletion of SMN1 exon 7. Besides, improvements in several methods such as optical design will undoubtedly lead to higher sensitivity of SMN-selective biosensors. Moreover, the biosensors developed for the diagnosis and monitoring of SMA can be further improved with the use of novel materials with ideal structures in the design, with new methodologies for antibody immobilization and stabilization,

miniaturization of the existing sensors, lowering the required sample volume and analysis time, replacing hazardous solvents with greener alternatives, etc. In addition, personalized medicine has increased the demand for customized health services such as biosensors for screening and diagnosis according to the specific genetic structure of patients. A biosensor that can provide targeted approaches for genetic diseases such as SMA can meet the demands in this field, by providing superior analytical capabilities and ease of analysis without any training. Further research on biosensors can have a direct impact on SMA diagnosis, management and monitoring of individual health conditions, such as disease severity and treatment response. Besides, lowering the analysis costs associated with SMA diagnosis and monitoring with the help of novel biosensors will certainly enable the widespread application of these methods in resource-limited regions, ultimately allowing the earlier diagnosis of SMA in these populations, leading to higher treatment success and lower associated health costs. These novel biosensors can also play a critical role in the treatment planning of the disease, and improve the quality of life for people living with SMA.

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