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REVIEW PAPER

# Biosensors, Recent Advances in Determination of BDNF and NfL

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## Abstract

Key biomarkers such as Brain Derived Neurotrophic Factor (BDNF) and Neurofilament light chain (NfL) play important roles in the development and progression of many neurological diseases, including multiple sclerosis, Alzheimer's disease, and Parkinson's disease. In these clinical conditions, the underlying biomarker processes are markedly heterogeneous. In this context, robust biomarker discovery is of critical importance for screening, early detection, and monitoring of neurological diseases. The difficulty of directly identifying biochemical processes in the central nervous system (CNS) is challenging. In recent years, biomarkers of CNS inflammatory response have been identified in various body fluids such as blood, cerebrospinal fluid, and tears. Furthermore, biotechnology and nanotechnology have facilitated the development of biosensor platforms capable of real-time detection of multiple biomarkers in clinically relevant samples. Biosensing technology is approaching maturity and will be deployed in communities, at which point screening programs and personalized medicine will become a reality. In this multidisciplinary review, our goal is to highlight clinical and current technological advances in the development of multiplex-based solutions for effective diagnosis and monitoring of neuroinflammatory and neurodegenerative diseases.

## Graphical Abstract

The trend in the detection if BDNF and NfL.


**Keywords** Biosensors · Brain · Ultra-sensitive · BDNF · NfL · CNS · Neurological diseases

Extended author information available on the last page of the article

**Abbreviations**

| | |
|---|---|
| BDNF | Brain-derived neurotrophic factor |
| ELISA | Enzyme-linked immunosorbent assay |
| IMR | Immunomagnetic reduction |
| EIS | Electrochemical impedance spectroscopy |
| IDE | Interdigitated electrode |
| MWCNTs | Multi-walled carbon nanotubes |
| LOD | Limit of detection |
| LOQ | Limit of quantitation |
| CV | Cycling voltammetry |
| DPV | Differential pulse voltammetry |
| SPE | Screen-printed electrode |
| NfL | Neurofilament light chain |
| SMA | Single-molecule array |
| MBs | Magnetic microbeads |
| PEC | Photoelectrochemical |
| PtWP | Woodpile-like Pt nanowire |
| EGOFET | Electrolyte-gated organic field-effect transistor |
| IDE | Interdigitated electrode |
| 3,5- DClPDT | 3,5-Dichlorophenyl diazonium tetrafluoroborate |
| Na-DEDTC | Sodium diethyldithiocarbamate |

## Introduction

Brain-derived neurotrophic factor (BDNF) belongs to a family of neurotrophins that play imperative roles in the survival and differentiation of developing neuronal populations (Park and Poo 2013; Zagrebelsky and Korte 2014). In the adult brain, BDNF maintains high levels of expression and regulates both inhibitory synaptic transmission and excitatory and activity-dependent plasticity (Park and Poo 2013). It profoundly affects neuronal physiology and morphology, promotes synaptic stabilization and neurite germination, and increases long-term potentiation. BDNF synthesis and release are regulated by neural activity and are consistent with BDNF's role as the main mediator of activity-dependent neuroplasticity (Kavalali and Monteggia 2020). Recent information recommends that BDNF may also be associated with spontaneous, activity-independent transmission (Zagrebelsky and Korte 2014; Kavalali and Monteggia 2020). BDNF is a neurotrophic signaling molecule related to synaptic plasticity, neuronal growth, synapse maturation during development, and axonal targeting (Bramham and Messaoudi 2005). BDNF is related to various of mental disorders, including: bipolar disorder, deep depression, anxiety disorders, and other psychiatric disorders associated with exposure to stressful conditions (Hall et al. 2011; Akhtar et al. 2018). Therefore, alterations in BDNF blood levels due to genetic and environmental factors are increasingly associated with several psychiatric disorders (Han et al. 2019; Khairy and Attia 2021). Decreased serum BDNF occurs in depression, bipolar disorder, eating disorders, and schizophrenia, and there is evidence that drug treatment for these disorders upregulates BDNF levels (Han et al. 2019; Khairy and Attia 2021). Low serum BDNF levels may also be associated with depression-related personality traits in healthy subjects, suggesting their potential role as risk markers (Lang et al. 2004; Han et al. 2019). Additionally, neurofilament light chain protein (NfL, approximately 68 kDa) is one of many proteins in the neuronal cytoskeleton (Zhou et al. 2017; Lin et al. 2018). The protein is released upon axonal damage in the central nervous system (CNS) and can be detected in serum, cerebrospinal fluid (CSF), and EDTA-plasma after passage through the blood–brain barrier (Lin et al. 2018). NfL levels in serum/plasma are several times lower than in CSF, but the correlation is strong. Plasma levels are lower than serum levels (approximately 10 percent lower) (Zhou et al. 2017). NfL levels in serum/plasma can thus be used as a marker of CNS neuronal degeneration. NfL levels are an unspecific marker of neuronal degeneration that does not reveal the underlying cause (Gaetani et al. 2019; Alirezaei et al. 2020). The level of NfL is extremely useful in monitoring the progression of demyelinating diseases. The reference ranges are broad because the NfL level varies greatly between individuals in the general population (Gaetani et al. 2019; Yuan and Nixon 2021). Therefore, precise monitoring of BDNF and Nfl in cerebrospinal fluid (CSF) and other physiological fluids has the potential to discover important clues for the development of therapeutic and diagnostic approaches for psychiatric diseases and neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease (Scalzo et al. 2010; Hosang et al. 2014). To evaluate the physiological significance of human salivary BDNF, research work has optimized a sensitive and specific enzyme-linked immunosorbent assay (ELISA) (Vikkula 2013; Kuhle et al. 2016a, b). Our goal is to provide the latest and comprehensive insights on biosensing techniques in the detection of BDNF and Nfl, focusing on the potential potential of biosensing in the determination of a wide range of disease biomarkers and targets.

## Plasma Levels of BDNF and NfL

In healthy volunteers, mean plasma BDNF levels ranged from 92.5 pg/mL (8.0 to 927.0 pg/mL). It was higher in women and decreased with age in both men and women (Zhang et al. 2007). BDNF is spread over numerous brain areas and helps neurons survive, support, and function. Other sources of BDNF include the gastrointestinal tract, lungs, heart, spleen, and liver. Apart from that, BDNF is known to be expressed in vascular smooth muscle cells, fibroblasts, and thymic stroma (Raznahan et al. 2009). The

results confirmed that BDNF levels in the colon, bladder, and lungs were higher than those in the skin and brain (Lommatzsch et al. 2005). Positive associations have been reported between blood levels of BDNF and low-density lipoprotein (LDL) cholesterol, diastolic blood pressure, total cholesterol, body weight index, adipose tissue mass, and triglyceride. It has been recommended that women with low plasma BDNF levels are at increased risk of death (Golden et al. 2010). The observation supports that a significant decrease in plasma levels of BDNF in women was associated with age and weight gain (Jung et al. 2011). Plasma NfL levels were positively associated with age ($r = 0.355$, $p < 0.001$) and negatively with global cognition ($r = -0.355$, $p < 0.001$) in all subjects (Zhou et al. 2017).

## Methods in the Detection of BDNF and NfL

Given the above references to those different roles, there is increasing interest in achieving precise and quantitative detection of different BDNF protein morphologies. Because BDNF is a secretory protein, it is found both intracellularly and extracellularly (Fletcher et al. 2018). BDNF is a diversity of astrocytes (primarily due to trans-cytosis), neurons, microglia, vascular endothelial cells, monocytes, male submandibular glands, smooth and striatum muscles, peripheral pericytes, etc. (Kojima et al. 2020; Keifer 2021). The enzyme-linked immunosorbent assay (ELISA) is an immunological assay frequently used to quantity proteins, antibodies, antigens, and glycoproteins in biological samples. Some examples include pregnancy tests, diagnosis of HIV infection, and quantity of cytokines or soluble receptors in cell supernatant or serum (Konstantinou 2017). The working principle of ELISA is presented in Fig. 1.

As revealed in Fig. 1, high sensitive ELISA method using an antibody with high specificity and detection sensitivity having an epitope on the *N*-terminal side of mBDNF. The antibody used in these products has very low cross-reactivity with proBDNF, allowing specific and sensitive detection of mBDNF.

To evaluate the physiological significance of human salivary BDNF, a specific and sensitive ELISA was developed appropriately (Mandel et al. 2011). To determine the relationship between salivary BDNF levels and other biological characteristics, an ELISA method was examined. Results showed no association between BDNF levels in saliva and other biological characteristics (Mandel et al. 2011). Western blotting showed BDNF levels were reduced in treated animals compared to control animals (Cassano et al. 2006). Western blotting is an experimental technique for detecting specific proteins in blood or tissue samples. This method uses gel electrophoresis to separate the proteins in the sample. The separated protein is transferred from the gel to the surface of the membrane (Zhang et al. 2018). In an original study, western blotting evaluated the secretion of met BDNF and val BDNF in serum. Finding revealed equal amounts of proteins were loaded in each lane so, mBDNF and vBDNF proteins are expressed at an equal level and treated in the

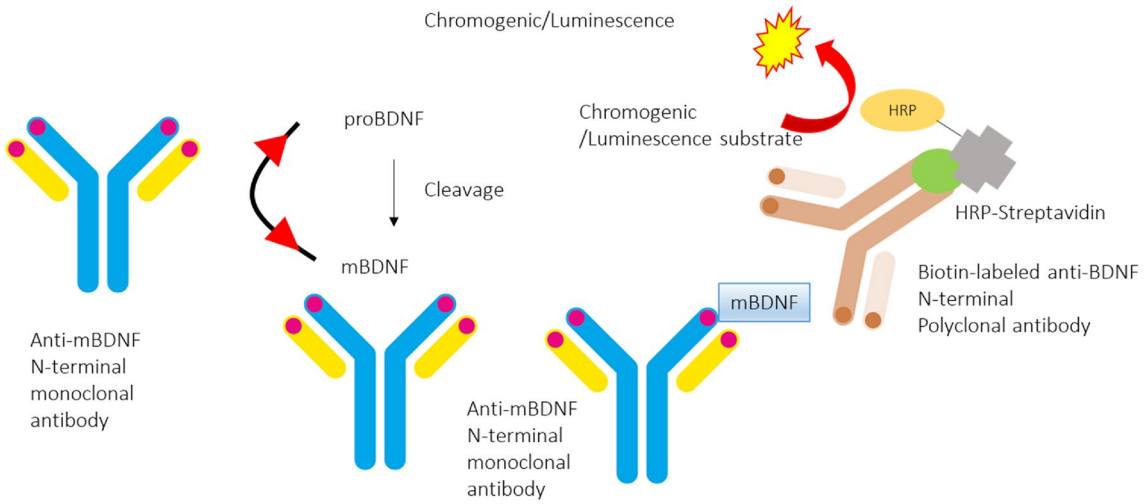


**Fig. 1** Working principle of ELISA, BDNF is expressed as a precursor BDNF (proBDNF), transported to secretory sites and processed into mature BDNF (mBDNF). Previously, the precursor proBDNF was thought to have no physiological function, but it was recently revealed that it has a completely different physiological function from mBDNF. mBDNF induces long-term potentiation (LTP) through TrkB and promotes neurogenesis and neurodevelopment. On the other hand, proBDNF induces long-term depression (LTD) through the low-affinity receptor p75NTR and promotes neuronal apoptosis. Variations in this effect with different treatments suggest the existence of highly complex regulatory mechanisms of BDNF in the nervous system. Therefore, methods to accurately measure mBDNF are needed to elucidate the mechanisms of various psychiatric disorders

same method (Egan et al. 2003). Targeting histone deacetylation for recovery of maternal deprivation-induced variations in AKAP150 expression and BDNF in the ventral tegmental area (VTA) were determined by the western blotting (Shepard et al. 2018) (Table 1).

## What Is the Biosensor Methodology?

The term "biosensor" is an abbreviation for "biological sensor". The device consists of biological elements such as enzymes, antibodies, nucleic acids, and a transducer. The biological element interacts with the analyte under test, and the transducer converts the biological response into an electrical signal (Belkhamssa et al. 2016; Coulet 2019). Depending on the application, biosensors are also known as immunosensors, genosensor. The commonly cited definition of the biosensor is "a chemical sensing device in which biologically obtained detections are combined with a transducer to enable the quantitative development of several complex biochemical parameters. (Belkhamssa et al. 2016; Coulet 2019). Figure 1, presents the biosensor methodology.

Mandatory use of biosensors has become of paramount importance in biomedical drug discovery, food safety standards, and environmental monitoring. Biosensors have been classified based upon numerous outlooks; however, the most generally used classification relies on two factors: the transducer and the bioreceptor (Thévenot et al. 2001; Alhadrami 2018).

As shown in Fig. 2, three major transducer-based groups have been developed over the last two decades, allowing different bioreceptors to be applied to different biological targets.

With cancer morbidity and mortality increasing globally, science leveraging technological advances to combine nano/biotech insights with data science to enable better diagnoses

**Table 1** Methods in the detection of BDNF and NfL

| Techniques | Biomarker | LOD/sensitivity | Findings and significances | References |
|---|---|---|---|---|
| ELISA | BDNF | ~15 pg/µl | Developed system was based on colorimetric detection. Also, the potential utilization of chemiluminescence based detection technique was approved successfully | Vikkula (2013) |
| ELISA | BDNF | 618 pg/mL | There was no association between salivary BDNF levels and the other biological characteristics examined | Mandel et al. (2011) |
| Western blotting | BDNF | – | – | Cassano et al. (2006) |
| Western blotting | BDNF | 10 µg | Levels of mBDNF proteins in mBDNF-GFP transfected cells (both the pro- and mature forms) were detectable | Egan et al. (2003) |
| Western blotting | BDNF | – | – | Shepard et al. (2018) |
| ELISA | NfL | 78.0 pg/mL | Finding indicated SMA to be more sensitive than ELISA or the ECL assay | Kuhle et al. (2016a, b) |
| ELISA | NfL | 10 µg/L | ELISA, which quantifies NfL from human CSF, has revealed the difficulty of preparing accurate and consistent standards | Petzold et al. (2010) |
| ELISA | NfL | 15 nM | These results show a noble analytical performance of the novel ELISA for quantification of NfL concentrations in the CSF | Gaetani et al. (2018) |
| ELISA | NfL | 1000 pg/mL | Developed method was promising not only for screening dementia but also for differential diagnosis | Liu et al. (2020) |
| IMR | NfL | 0.18 fg/mL | – | Chung et al. (2020) |
| SMA | NfL | 14 ng/L | Plasma NFL is associated with AD diagnosis and with cognitive, biochemical, and imaging hallmarks of the disease | Mattsson et al. (2017) |

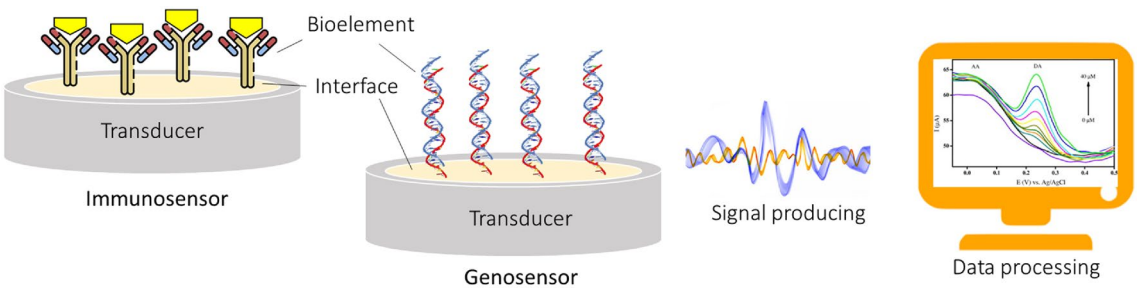


**Fig. 2** Biosensor methodology

and reduce cancer misdiagnosis and there is an urgent need to limit late diagnosis. Biosensors as nanobioelectronics are expected to be important tools to advance the detection of new specific biomarkers that are molecularly relevant to specific types and stages of human cancer (Hossain et al. 2017; Lee et al. 2020). In the early stages of cancer, biomarkers are only present in trace amounts. Therefore, the reliability and sensitivity of diagnostic tests are of great importance. Ideally, biomarkers should be detected with highly sensitive and minimally invasive medical procedures (Topkaya et al. 2016). Currently, clinical cancer detection is mainly done by imaging methods such as mammography, X-ray, magnetic resonance imaging, computed tomography, endoscopy, and ultrasound. Although these methods produce somewhat acceptable results, they suffer from weaknesses such as high cost, low sensitivity and specificity, and false-positive and false-negative results due to biosensor properties. Most of these vulnerabilities are covered (Topkaya et al. 2016) (Fig. 3).

## Nanomaterials in Biosensors

Nanomaterials are now undertaking rapid expansion due to their potential applications in catalysis, nanoelectronics, structural components, magnetic data storage, biomaterials, biosensors, and more. The use of NPs, nanotubes, nanowires, etc. in biosensor investigative devices is under consideration. As the properties of nanomaterials and their nanoscale dimensions' advance, new bio-devices (smart biosensors) are emerging that can detect trace concentrations of desired analytes. Nanomaterials are commonly used as transducer materials, an imperative part of biosensor development. Engineered nanomaterials offer higher electrical conductivity, are nanoscale in size, can be used to amplify desired signals, and are compatible with biomolecules. For example, carbon materials can be used to bind biomolecules (DNA, enzymes, antibodies, cells, etc.). It has been found that the use of nanomaterials can lead to improved biosensor performance, such as increased sensitivity and numerous orders of magnitude lower detection limits. Nanostructured materials exhibit enlarged surface-to-volume ratio, mechanical strength, higher chemical activity, electrocatalytic properties, and improved diffusivity. Nanomaterials are anticipated to play an important role in the high performance of biosensors. Biocompatibility of nanomaterials is an important factor in the development of biosensors when studying biomolecules such as bacteria, viruses, and DNA. In this chapter, we discuss nanomaterials with various applications for biosensor development. Some important and mostly used nanoparticles are presented in Fig. 4.

In summary nanomaterials became important components in biosensing methodology since they obviously improve the performances in terms of sensitivity and detection limits down to single molecules detection.

## Developed Biosensors for BDNF Detection

Quantification of endogenous BDNF can be performed with a ELISA, and despite frequent use of BDNF ELISA kits the working issues regarding quantities of BDNF concentrations in the blood and mainly in the brain have not been studied in depth, and only newly the reproducibility, accuracy, and influence of various storage conditions on blood BDNF measurements have been described (Marell et al. 2019; De Sá et al. 2023). One major problem is that diverging serum, plasma, and whole blood BDNF results have been reported in humans. Unfortunately, these divergent results from human studies also exist in several preclinical studies, despite using the same rat strain, extraction buffer, and dilutions (Marell et al. 2019; De Sá et al. 2023). This calls for a systemic approach, as inappropriate use of the ELISA kit and handling of the biological material are possible

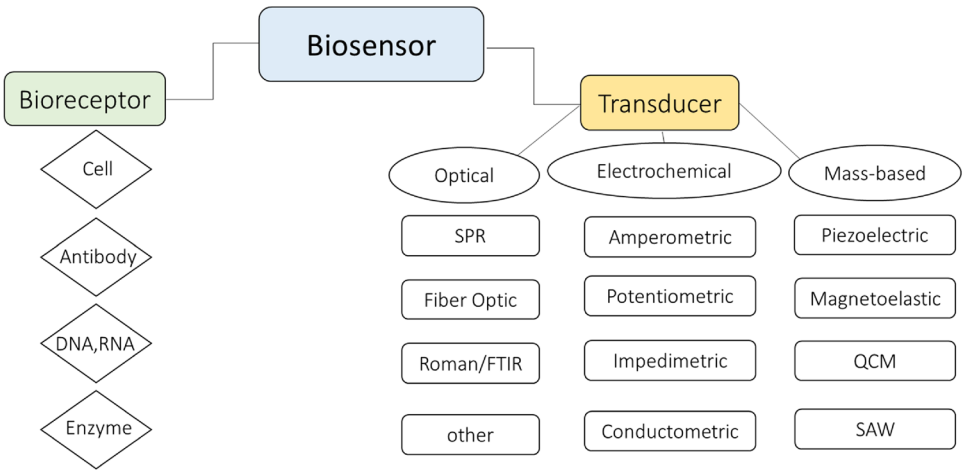


**Fig.3** Biosensor classification

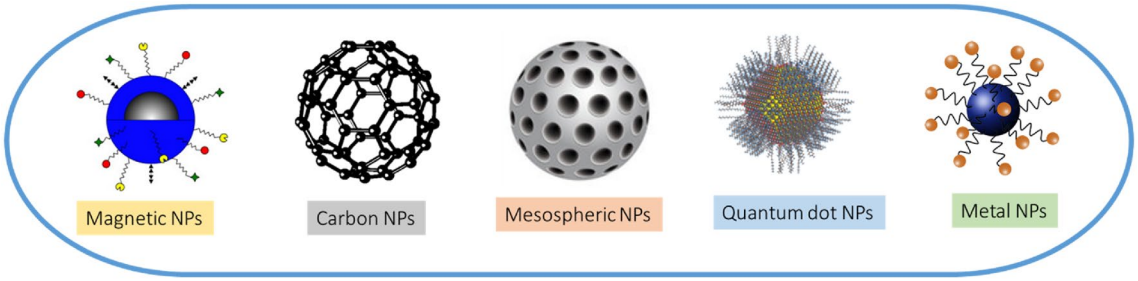


**Fig.4** The most important biomaterial used in biosensor technology

explanations for the divergent results mentioned above. Therefore, as mentioned earlier, in recent years, biosensors are being developed as suitable tools for BDNF detection, and some of the most important ones have been examined in the following.

The biosensor device was manufactured for use with a small amount of blood that can be collected from a finger puncture wound to detect BDNF. Specifically, the presented device is a polymer-based chip with a nanoporous wrinkled gold film that acts as an electrode/detection layer, enabling electrochemical detection of BDNF in plasma. Increasing the BDNF concentration (0.1–2.0 ng/mL) resulted in a significant difference in redox current. Biosensors are ideal for generating signal displays in seconds and achieving multiplexing (Bockaj et al. 2018). An interdigital microelectrode (IME) immunosensor coated with anti-BDNF antibody and anti-BDNF in a polydimethylsiloxane (PDMS) -based microfluidic channel chip was developed to detect BDNF in the brain of mice. The platform detected BDNF from microliter liquid samples, even at femtogram/mL concentrations, demonstrating higher selectivity than other growth factors. By Using this methods examined whether BDNF is detectable from periodical collection of cerebrospinal fluid microdialysate, tasted every 10 min from the hippocampus of mice through the context-dependent fear-conditioning test (Yoo et al. 2016). An ultrasensitive dual probe immunosensor was proposed to monitor of nicotine induced-brain derived neurotrophic factor released from cancer cells. In this study the working probe was created by covalently immobilizing capture anti-BDNF (Cap Ab) on the gold nanoparticles (AuNPs)/conducting polymer composite layer. The applied probe was modified by drop casting a bioconjugate particles composed of conducting polymer self-assembled AuNPs, immobilized with detection anti-BDNF (Det Ab) and toluidine blue O (TBO). Created biosensor showed acceptable linearity and high sensitivity at optimized conditions (Akhtar et al. 2018). BDNF dysregulation is generally associated with several neurological and psychiatric disorders such as schizophrenia, and the available evidence is that BDNF is also strongly associated with Parkinson's disease and Alzheimer's disease. The BDNF target sequence was detected with a capture probe attached to an aluminum microcomb electrode on the surface of a silicon wafer. A capture target reporter sandwich assay was performed to improve the detection of BDNF targets. The LOD was investigated to be 100 aM. Input of a reporter sequence at concentrations > 10 aM enhanced the detection of the target sequence by enhancing changes in the generated currents (Li et al. 2021a, b).

A 3D aptersensor based on bio-layer interferometry (BLI) technology for early detection of glaucoma, an irretrievable disease that causes blindness, followed by rapid and sensitive detection of BDNF was settled recently. The developed 3D aptersensor has made it possible to batch convert a small amount of biomarker BDNF binding into an optical interference signal in one step. The planned biosensor sjowed a broad range linearity 0.41 to 250 ng/mL BDNF, with a LOD of 0.2 ng/mL (Gao et al. 2022). An innovative immune microelectrode array was developed as a label-free electrochemical biosensor for the detection of BDNF in human serum. The examination results of performance (Fig. 5) showed that the engineered immune-platform had a good selectivity, stability, and the limit of detections for BDNF measurement and was applicable in neuroscience researche (Xu et al. 2017).

As revealed in the Fig. 5, the chitosan-thionine-multiwalled carbon nanotubes (CS-THI-MWCNTs) composite films as the bio-sensitive film are modified onto the MEA by electrochemical deposition and effectively adopted to immobilize anti-BDNF for the fabrication of electrochemical immune MEA.

A MIP-based synthetic receptor selectively bind to a clinically relevant protein, such as BDNF. BDNF-MIP was produced directly on the screen-printed electrode (SPE) by surface initiation control/living radical photopolymerization. The resulting BDNF-MIP/SPE electrochemical sensor was able to detect BDNF-acceptable LODs in the presence of interfering HSA proteins and identify BDNF from structural analogs such as the neurotrophic factors CDNF and MANF (Kidakova et al. 2019). Molecularly imprinted polymers (MIPs), which are entirely synthetic materials with antibody-like ability to bind and discriminate between molecules, exhibit

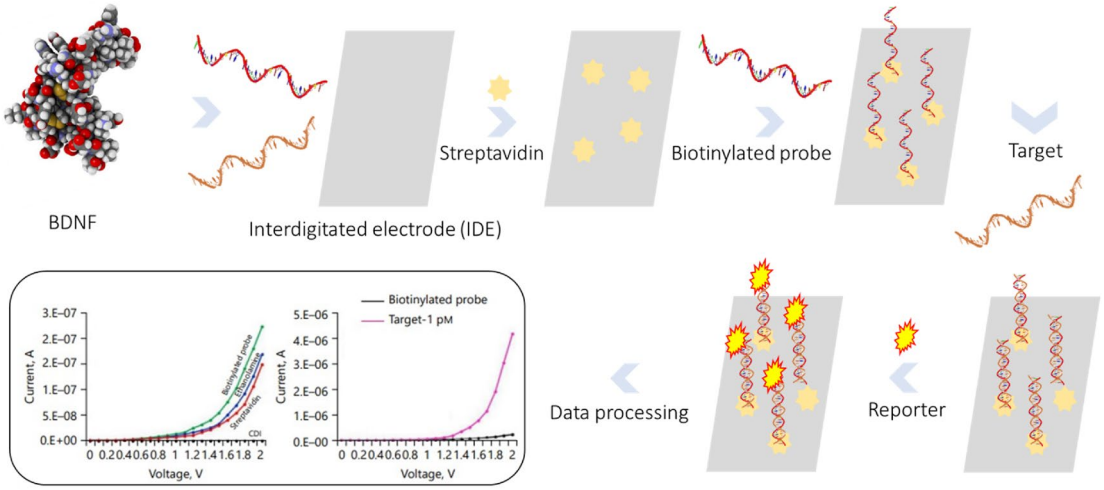


**Fig. 5** Developed immunosensor for detection of BDNF, reproduced from Ref. (Li et al. 2021a, b)

enhanced stability and reduced fabrication cost compared to biological receptors. MIP based immunosensor Fig. 6, was advanced for sensitive determination of BDNF directly on a screen-printed electrode (SPE) (Kidakova et al. 2019) (Fig. 7).

The developed approach may be a promising method for developing innovative PoC diagnostic devices for early diagnosis and/or therapeutic monitoring of neurological diseases (Kidakova et al. 2019).

## Developed Biosensors for NfL Detection

Neurofilament light chains are intra-axonal structural proteins released into both CSF and blood fluids upon axonal injury, regardless of the cause (Gaetani et al. 2019; Varhaug et al. 2019). It is vague how blood levels of NFL correlate with neurodegeneration, but it is one of the most consistent plasma biomarkers of neurodegeneration (Gaetani et al. 2019; Varhaug et al. 2019). The use of blood samples is considered preferable to CSF samples, but the ELISA method has relatively low sensitivity, and because NfL

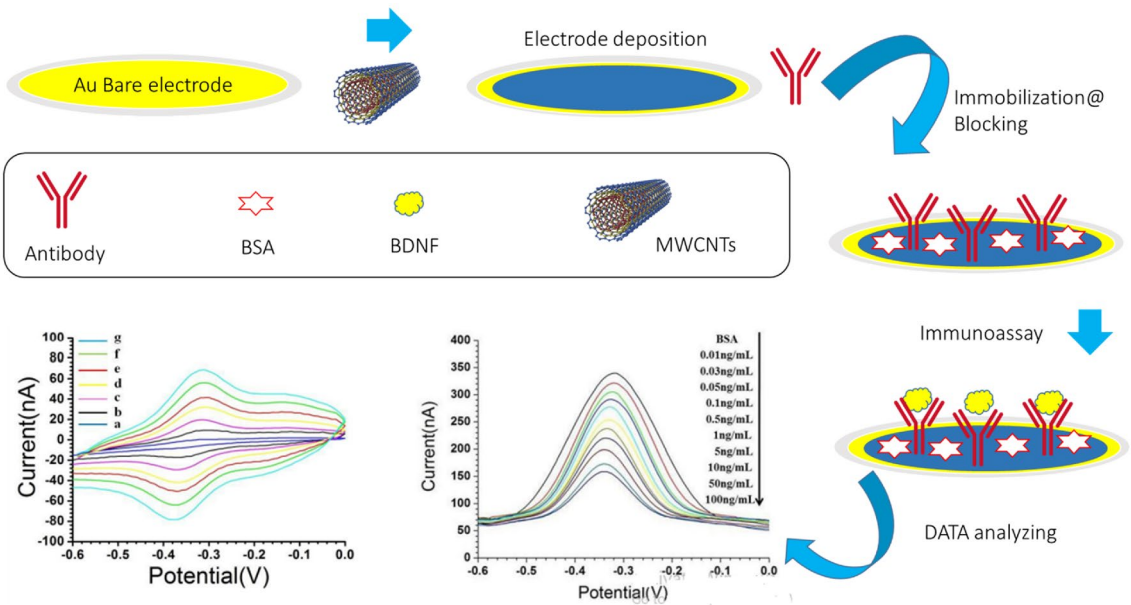


**Fig. 6** The electrochemical immune microelectrode array modification procedure, reproduced from Ref. (Xu et al. 2017)

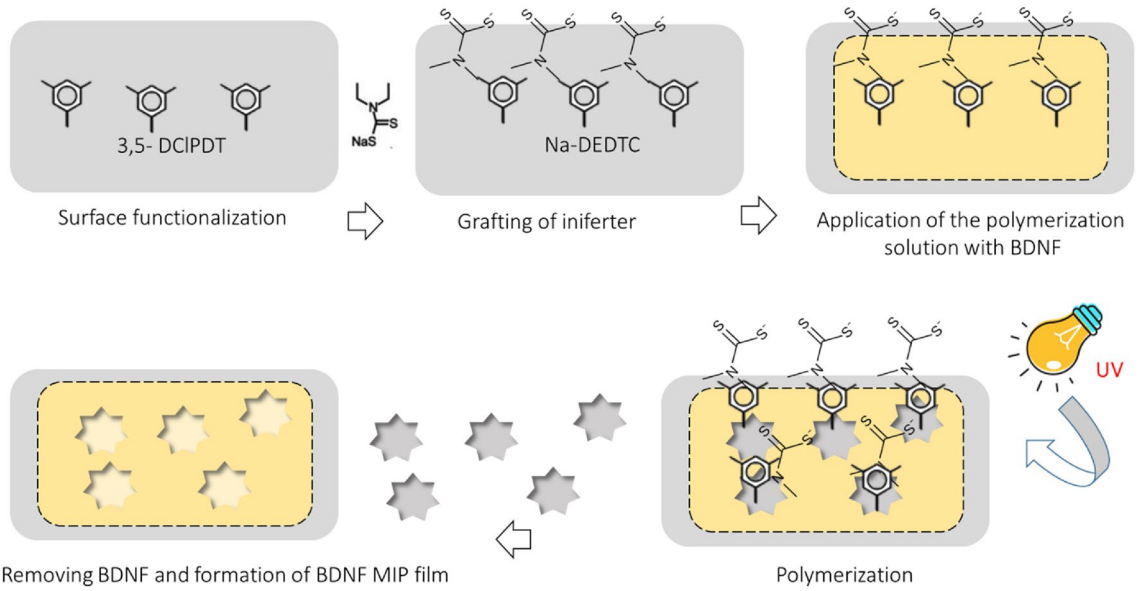


**Fig.7** Fabrication of the BDNF-MIP/SPE sensor, adapted from Ref. (Kidakova et al. 2019)

concentrations are lower in blood, these evaluations have been traditionally being limited to CSF samples. However, the biosensor platform is a new method with higher sensitivity so it can be reliably used to assess NfL levels in blood (Bacioglu et al. 2016). A few studies have used the more sensitive electrochemiluminescence (ECL) technologies to evaluate serum NfL levels. These studies have shown that NfL measurements in the blood, as well as the CSF, can be used as biomarkers for diseases including MS (Kuhle et al. 2016a, b). In the following, some advanced biosensors for its detection are reviewed and introduced.

Glycidyl methacrylate (GMA) efficient monomer was applied to form thin polymeric films on the electrode surface and the anti-NfL antibody was immobilized via covalent linkage with GMA as a bio-recognition agent. The functional monomer (MAC) was synthesized through the reaction between L-cysteine and benzotriazole methacrylate (MA-Bt). The advantage of a developed immunosystem involves in its high recognition ability and simplicity. Moreover, in this work acceptable LOD and LOQ as 5.21 ng/L and 15.79 ng/L has been reported for the determination of NfL concentration (Özgür et al. 2020). Magnetic microbeads (MBs)-based electrochemical bioplatform established for the detection of NfL-based on a sandwich type immunoassay including an HRP-secondary antibody to enzymatically label the detector antibody on the surface of commercial MBs functionalized with carboxylic acids. In this method amperometric transduction is carried out at SPCEs upon deposition of the magnetic bioconjugates on the working electrode surface using the $H_2O_2$/hydroquinone (HQ) system. Observed analytical performance is suitable for determining NfL in brain tissues and plasma from AD patients using fast and straightforward methods and needing low samples (Valverde et al. 2021) (Fig. 8).

To assemble a bias-free PEC detection system, water-oxidizing with an iron oxyhydroxide-deposited bismuth vanadate DA2-PtWP biocathode ($FeOOH/BiVO_4$) photoanode. Proposed biosensor was employed for determination of NfL in blood samples appropriately. In this research the platform was assembled by incorporating molybdenum (Mo) dopant and iron oxyhydroxide (FeOOH) ad-layer into the $BiVO_4$ photoelectrode and employing a signal amplifier formed by horseradish peroxidase (HRP)-triggered oxidation of 3,3'-diaminobenzidine (DAB) (Kim et al. 2020). A lanthanide-doped MOFs ($La/UiO\text{-}66\text{-}NH_2$) material is pyrolyzed and synthesized as an ancestor to obtain a novel MOFs-derived material ($ZrO_2@La_2O_3$) with exceptional electrochemical properties and effective immobilization of antibodies. $ZrO_2@La_2O_3$ is employed as an electrode modification material to build a label-free electrochemical immunosensor. The system created was cheap, easy to manufacture, and easy to see. This could meet her future NfL assessment needs and become an efficient platform that would be of great help in the clinical diagnosis and treatment of neurodegenerative diseases (Li et al. 2022). Organic semiconductor molecule Y6 was utilized in the construction of a novel immunosensor for ultra-sensitive detection of NfL. The effective demonstration of Y6 in this research for PEC recognition of NFL paved an original approach for the application of organic semiconductors in PEC sensing field (Chen et al. 2021).

An electrolyte-gated organic field-effect transistor (EGOFET) was settled for sensitive detection of NfL in sub-pM levels. The selective, label-free, and fast response

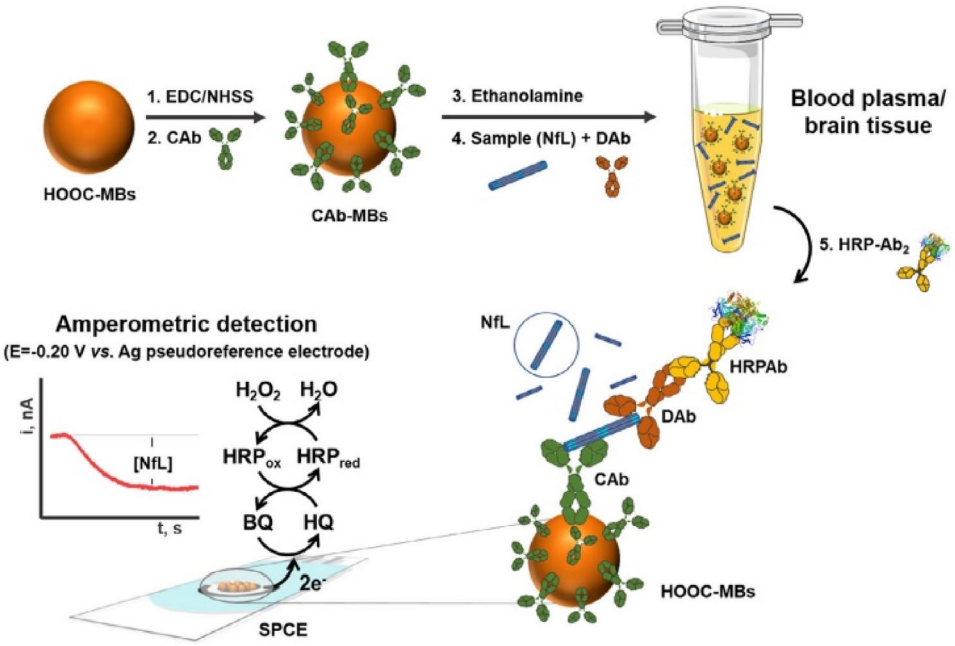


**Fig. 8** Schematic of the sandwich immunoassay approach implemented in MB and amperometry detection in SPCE for NfL determination using the H2O2/HQ system. Mouse NfL capture mAb (capture antibody, used as CAb), rabbit neurofilament L detection antibody (detection antibody, used as DAb), anti-rabbit IgG HRP-binding antibody (labeled secondary antibody, used as HRP-Ab2) with permission (No: 5358781098247) from Ref. (Valverde et al. 2021)

makes this EGOFET biosensor a suitable device for monitoring and diagnosing neuronal injuries through detecting NF-L in physio-pathological ranges. Advanced system worked based on the EGOFET construction and can quantify NF-L down to sub-pM levels; thanks to modification of the device gate with anti-NF-L antibodies imparted with potentially controlled orientation (Solodka et al. 2022). An innovative electrochemical immuneplatform for the determining of NfL was advanced based on sandwich immunocomplexes labeled with HRP (b) and amperometric technique at SPCEs. Planned biosensor showed excellent selectivity and sensitivity and was usable for the determination of some biomarkers such as NfL, Tau, and p-Tau in human plasma (Valverde et al. 2022). Gold-nanourchin complexed silicon dioxide-probe on gap-fingered IDE surface was established for NfL determining using current–volt measurement. Created biosensor Fig. 9, demonstrated excellent analytical performance and was applicable to quantity the level of NfL and leads to determine the condition with PD (Li et al. 2021a, b).

As illustrated in Fig. 9, immunosensor was generated by current–volt measurement on gap-fingered interdigitated electrode with silicon dioxide surface to determine NfL level. To improve the detection, anti-NfL antibody was complexed with gold-nanourchin and immobilized on the sensing electrode (Li et al. 2021a, b) (Fig. 10).

As discussed in the previous section, biosensors can be a useful alternative to older methods such as ELISA in detecting biomarkers associated with neurological diseases. Tables 2, and 3 summarize the details of biosensors developed for the detection of BDNF and NfL.

## Conclusions

This review tried to collect all recent trends in advanced biosensors for the detection of BDNF and NfL. Presented biosensors have ultrasensitive detection limits, wide range linearity, and acceptable selectivity and stability in some cases. The main challenge in biosensor expansion needs to keep improving these technologies. For instance, the selection of enzymes, electrodes, and nanomaterials are important factors in the development of the biosensor because they must have similar operating conditions (concentration, temperature, pH,). Future work should focus on understanding the mechanism of interaction between the nanomaterials and enzymes and on the fabrication of new materials with electrode surface that could be applied in clinical diagnosis. As a result, it is recommended that the following items be considered in future studies. (A) Development of biosensors capable of detecting different critical neuroscience-related biomarkers such as BDNF, NfL, Tau protein, and Aβ. (B) Improving biosensor functional stability in various laboratory conditions. (C) Dedicated effort to develop more affordable, simple, and user-friendly biosensors with a higher sensitivity approach, less sample volume, and fewer pre-treatment steps. (D) Concentrate on miniaturization, such as microfluidic device fabrication and the integration of nanomaterials with other biosensor components for in situ heavy metal detection. (E) Using nanotechnology to integrate artificial intelligence

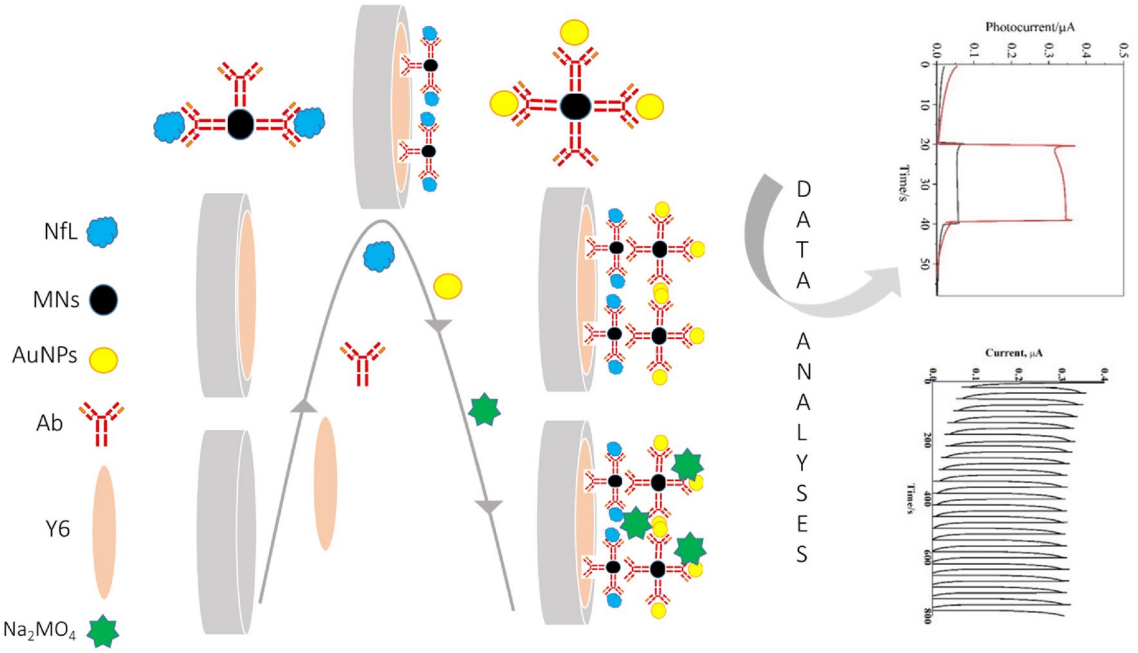


**Fig. 9** PEC response of Y6 modified electrode in the absence and presence 10 mM $H_2O_2$. Stability of the photocurrent intensity of the modified electrode with continuous on/off light irradiation cycles, adapted from Ref. (Chen et al. 2021)

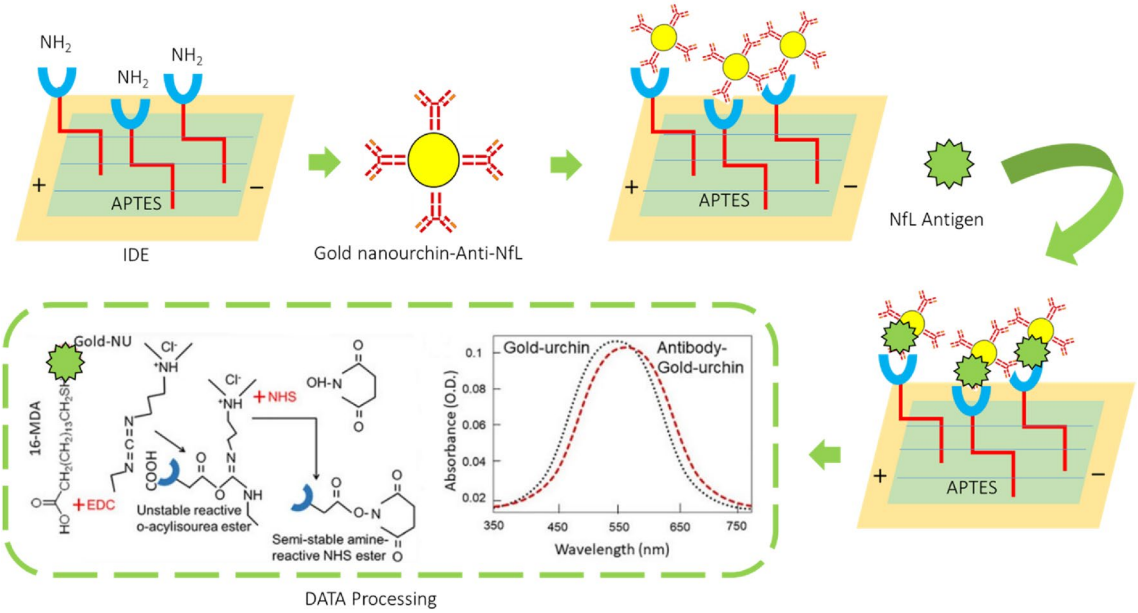


**Fig. 10** A scheme for surface functionalization and NfL detection in a gold nanourchin antibody-modified IDE. It is an immobilization process using the APTES linker and is detected by the NfL. Gold nanourchin binding process with antibodies. Gold nanourchin was first bound with 16-MDA, then he was activated with EDC and NHC, and then the antibody reacted. Ultraviolet–visible spectroscopic analysis. The spectra before and after attachment of the antibody to gold nanourchin are shown, reproduced from Ref. (Li et al. 2021a, b)

**Table 2** Developed biosensors for detection of BDNF

| Platform | Technique | Matrix | NPs | Model | Linear range | LOD | References |
|---|---|---|---|---|---|---|---|
| Electrochemical/ Immunosensor | DPV | Plasma | AuNPs | Human | 0.1–2.0 ng/mL | 0.2 ng/mL | Bockaj et al. (2018) |
| Electrochemical/ Immunosensor | Impedance spectra | Brain | – | Mice | 10 fg/mL to 10 ng/mL | – | Yoo et al. (2016) |
| Electrochemical/ Immunosensor | EIS | Cancer cells | AuNPs | Human serum | 100 to 450 µg/mL | 1.81 ± 0.012 pg/mL | Akhtar et al. (2018) |
| Electrochemical/ aptasensor | – | Glaucoma | – | Human serum | 0.41 to 250 ng/mL | 0.2 ng/mL | Gao et al. (2022) |
| Electrochemical/ Immunosensor | CV, DPV | – | MWCNTs | Human serum | 0.01 ng/mL to 100 ng/mL | 5 pg/mL | Xu et al. (2017) |
| Electrochemical/ Immunosensor | MIP, CVs, DPV | Neurological diseases | SPE | Human serum | 0.01 to 0.06 ng/mL | 6 pg/mL | Kidakova et al. (2019) |

**Table 3** Developed biosensors for detection of NfL

| Platform | Technique | Matrix | NPs | Model | Linear range | LOD | References |
|---|---|---|---|---|---|---|---|
| Electrochemical/Immunosensor | CVs, EIS | Parkinson | GMA | Human | 1 to 50 µg/L | 5.21 ng/L | Özgür et al. (2020) |
| Electrochemical/Immunosensor | CVs, EIS | Brain | MBs | Plasma | 10 – 5000 pg/mL | 3.0 pg/mL | Valverde et al. 2021) |
| PEC | photochemical | Blood | Pt | Human | 1 ng/mL | 38.2 fg/mL | Kim et al. (2020) |
| Electrochemical/Immunosensor | CVs, DPV | Plasma | $ZrO_2$@$La_2O_3$ | Human | 0.05 ~ 200 ng/mL | 0.012 ng/mL | Li et al. (2022) |
| PEC | – | Serum | $Fe_3O_4$/AuNPs | Human | 5 pg/mL to 10 ng/mL | 1 ng/mL | Chen et al. (2021) |
| EGOFET | Adsorption | Biological | Gold gate | Serum | 100 fm to 10 nm | 30 fm | Solodka et al. (2022) |
| Electrochemical/Immunosensor | EIS | Plasma | CNTs | Human | 5–10 pg/mL | 10 pg/mL | Valverde et al. (2022) |
| Electrochemical/Immunosensor | Spectophotometry | Serum | AuNPs | Human | 100 fM to 1 nM | 100 fM | Li et al. (2021a, b) |

and biosensors to continue developing biomarker detection processes that are scalable, sensitive, and multifunctional.

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## Declarations

**Competing interest** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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