LASER-INDUCED GRAPHENE BASED ELECTROCHEMICAL SENSOR FOR SWEAT CORTISOL DETECTION: A LITERATURE REVIEW

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Abstract :- Psychological stress has a negative impact on health, triggering brain signaling pathways, leading to release of stress chemicals, most particularly cortisol. Cortisol, a steroid hormone, regulates the body's stress response. Measuring cortisol levels in biological samples is essential for detecting the physiology of stress and developing individualized treatment plans. Particularly in human sweat, electrochemical sensing has become a viable method for cortisol detection at the point of care. Nonetheless, there are still issues with electrochemical cortisol sensor development, including sample preparation, stability, sensitivity, and flexibility. This research investigates current open challenges and addresses potential future approaches in the field of cortisol detection while highlighting recent developments in electrochemical sensor technology.

1. INTRODUCTION

The stress hormone, cortisol, is the body's reaction to stress and has been linked to several physiological conditions. In recent years, non-invasive cortisol monitoring has attracted a lot of research and attention due to its potential for evaluating illnesses and problems linked to stress [1]. In recent years, the development of accurate non-invasive methodologies for cortisol concentration measurement has received a lot of attention [2].

Non-invasive cortisol sensing has the potential to monitor an individual's stress levels conveniently and continuously. Recent research has shown that electrochemical sensors may be used to detect cortisol reliably and quickly in sweat samples [3]. More study is necessary to improve sensitivity, optimize sensor performance and confirm their usefulness in clinical situations [4].

Studies on several non-invasive methods of cortisol sensing have been conducted, with an emphasis on electrochemical sensors that use biomarkers, found in human sweat. These sensors eliminate the need for invasive blood sampling by providing real-time monitoring capabilities and enable continuous cortisol level evaluation. The development of sweat biosensors enables non-invasive measurement of a wide range of analytes, including metabolites, nutrients, hormones, and medications [5], [6].

High levels of cortisol have been associated with an increased risk of cardiovascular disease and other adverse health outcomes, underscoring the clinical significance of accurate cortisol measurements [7]. Future directions may involve exploring novel biomarkers, conducting large-scale clinical trials to validate the reliability of non-invasive cortisol sensing devices, and

developing sensor design for increased sensitivity and specificity. Ultimately, the development of robust and user-friendly sensors could revolutionize the diagnosis and management of stress-related disorders, offering personalized insights into individuals' physiological responses to stress [8]. The fabrication process, consisting of formation of grapheme by means of laser carbonization, electrodeposition of metallic silver, chlorination of the deposited silver to form AgCl layer and electro analysis of the fabricated electrochemical cell were utilized for educational purposes [9].

Several studies have evaluated the performance of different electrochemical sensors for cortisol detection, considering factors such as stability. selectivity, and sensitivity. Advances in sensor design, including microfluidic integration and miniaturization, have contributed to enhanced sensing capabilities and portability, enabling potential applications in wearable devices for personal healthcare [10], [11].

This paper has highlighted the importance of non-invasive cortisol sensing in healthcare applications, particularly in assessing stress-related disorders and monitoring treatment effectiveness.

2. RECENT ADVANCES IN SWEAT CORTISOL SENSOR FABRICATION

[12] demonstrates a novel approach for fabricating a graphene-based three-electrode cell, offering a cost-effective alternative to commercial screen-printed electrodes commonly employed in electroanalytical applications. Conventional screen-printed electrodes present challenges such as complexity in fabrication and unknown ink composition, hindering electrode material modification studies. The authors employed laser-induced graphene (LIG) [13] as a low-cost fabrication technique for the required three electrodes. Optimization of laser power and rastering speed enhanced electrical conductivity and mechanical properties of the electrodes. Silver electrodeposition and subsequent anodization yielded silver chloride for stable open-circuit potential readings. Pseud-reference electrode performance was validated against a commercial counterpart, exhibiting comparable stability over a 16-hour period. Analytical application of the fabricated cell included dopamine concentration and coffee polyphenols determination. Future improvements may involve incorporating membranes for enhanced pseudo-reference electrode stability, as suggested by the authors.

In [14], a flexible immunosensing platform was developed for the selective detection of sweat cortisol. The platform involved the immobilization of gold nanoparticles (AuNPs) and multi-walled carbon nanotubes (MWCNTs) onto a stretchable polydimethylsiloxane (PDMS) [15], [16] substrate. Using electrochemical analysis such as differential pulse voltammetry (DPV), and cyclic voltammetry (CV) the electrochemical properties of the sensor were analyzed, and cortisol levels in human sweat were detected. With the combined conductivity of gold nanoparticles and mechanical stability of MWCNTs, the immunosensor exhibited excellent flexibility and electrochemical sensing performance, representing a linear detection range from 1 fg/mL to 1 mg/mL (R^2 = 0.995) and a detection limit of 0.3 fg/mL. The sensor detected cortisol levels accurately in human sweat, validated against a standard chemiluminescence

immunoassay (CLIA) method. This novel sensing platform shows a promising strategy for non-invasive cortisol monitoring in sweat and holds potential for wearable biosensor applications in health monitoring and clinical diagnosis.

A selective cortisol immunosensor was designed in [17] using a composite of MXene and multi-walled carbon nanotubes (MXene-MWCNTs). The fabrication process involved several steps, namely a three-electrode cell was screen-printed on polyethylene terephthalate (PET) film using silver/silver chloride ink for interconnection and reference electrode, followed by screen-printing of conductive carbon ink for counter and the working electrodes. The cell was then thermally cured at 70°C for an hour. MXene [18], obtained by etching Ti_3AlC_2 precursor in HF solution, was mixed with MWCNT and dispersed in deionized water utilizing ultra sonication. This dispersion was drop-casted onto the working electrode and dried. Functionalization of the electrochemical cell with a cortisol recognition layer involved crosslinking reagents N-(3-Dimethylaminopropyl)-N'-ethyl carbodiimide hydrochloride and N-Hydroxy succinimide (NHS), followed by immobilization of cortisol antibody and incubation with bovine serum albumin to prevent nonspecific protein binding. Characterization of MXene-MWCNT composite was conducted using scanning electron microscopy and transmission electron microscopy with energy-dispersive X-ray analysis. Electrochemical performance was evaluated via cyclic voltammetry (CV) in $[Fe(CN)_6]^{3-}$ /$[Fe(CN)_6]^{4-}$ solution, indicating increased active surface area and enhanced electrochemical conductivity compared to carbon-based electrodes. Immobilization of cortisol antibody reduced redox peak current, confirming successful antibody fixation. Electrochemical impedance spectroscopy (EIS) demonstrated reduced electron-transfer resistance with MXene-MWCNT assembly, while immobilization of cortisol antibody increased charge-transfer resistance. Optimization studies involved varying MXene-MWCNT mass ratio and concentration, as well as cortisol antibody concentration and incubation time. The optimized immunosensor exhibited differential pulse voltammetry (DPV) detection performance for cortisol concentrations ranging from 0.1 fg/mL to 1 µg/mL, demonstrating selectivity even in the presence of interferents.

Several studies have demonstrated the fabrication of highly sensitive and selective cortisol-detecting electrochemical sensors using graphene-based materials [19], [20]. involved utilizing a reduced graphene oxide (rGO) electrode as the sensing platform. By introducing a bifunctional protein interlayer, such as thermally denatured bovine serum albumin (d-BSA), the rGO electrode enables covalent anchoring of anti-cortisol antibodies while preventing nonspecific binding. This strategy enhances the sensor's sensitivity and selectivity, allowing for picomolar-range detection of cortisol with minimal cross-reactivity to analogs like aldosterone and progesterone. Characterization techniques such as electrochemical impedance spectroscopy (EIS) and scanning electron microscopy (SEM) have been employed to assess the morphology, conductivity, and sensing performance of graphene-based electrochemical biosensors. These sensors exhibit wide linear dynamic ranges (10 pM–100 nM) and low detection limits (~10 pM), making them suitable for detecting cortisol concentrations in human biological samples, including saliva. Validation studies involving spike recovery tests with human saliva samples containing interfering compounds have confirmed the reliability and accuracy of graphene-

based electrochemical biosensors for cortisol detection. Their practical applications extend to the diagnosis and monitoring of stress-related diseases, offering real-time and non-invasive cortisol level assessments. Graphene-based electrochemical biosensors hold great promise for sensitive and selective cortisol detection, offering a simple and efficient approach for diagnosing stress-related diseases. Further research focusing on optimization of sensor performance, validation in clinical settings, and exploration of novel sensing mechanisms will contribute to the advancement of this field and facilitate its translation into practical applications in healthcare monitoring.

In [21], a label-free electrochemical biosensor for ultrasensitive cortisol estimation was fabricated using polyaniline protected gold nanoparticles (PPAuNPs). Electrophoretic deposition was employed to uniformly deposit PPAuNPs onto a gold electrode. The PPAuNP/Au electrode was further modified by covalently binding cortisol-specific monoclonal antibodies (C-Mab) and blocking nonspecific adsorption with bovine serum albumin (BSA). Characterization techniques, including transmission electron microscopy, atomic force microscopy, and electrochemical impedance spectroscopy, were utilized to confirm PPAuNP formation and electrode modification. Cortisol detection was achieved through cyclic voltammetry and differential pulse voltammetry in phosphate buffer saline (PBS) solution. The biosensor demonstrated stability during repeated scans and exhibited repeatable redox peaks. Cortisol was accurately detected in the range of 1 pM–100 nM, with a sensitivity of 1.63 μA M^{-1}. Selectivity tests revealed the biosensor's specificity against BSA and 17-α-hydroxy progesterone. The study illustrates the potential of PPAuNP-based label-free electrochemical biosensors for sensitive cortisol monitoring in medical diagnostics and healthcare applications.

In [22], a wearable electrochemical impedimetric immunosensor with a microfluidic channel and chamber was developed for noninvasive point-of-care cortisol biomarker detection in human sweat. This type of sensor can be widely used within soft rehabilitation systems due to its flexible structure [23]. The sensor utilized a $Ti_3C_2T_x$ MXene-loaded laser-burned graphene (LBG) flakes 3D electrode network on a polydimethylsiloxane (PDMS) substrate. The LBG electrodes were initially fabricated on the PDMS substrate, with $Ti_3C_2T_x$ MXene [24] loaded to enhance conductivity and address inter-flake disconnection issues. The microfluidic system, comprising a 3D printed mold and PDMS, facilitated sweat collection and transport to the chamber. Characterization techniques including field emission scanning electron microscopy (FESEM) and X-ray photoelectron spectroscopy (XPS) confirmed successful $Ti_3C_2T_x$ MXene loading. Under optimized conditions, the $Ti_3C_2T_x$ MXene/LBG/PDMS-based patch cortisol immunosensor demonstrated a linear detection range of 0.01–100 nM and a detection limit of 88 pM. The study concludes that this strategy offers a well-suited and conformable approach for point-of-care cortisol biomarker detection. In this research, a wearable, microfluidic, and cost-effective patch was developed for noninvasive cortisol biomarker measurement in sweat. PDMS was employed for substrate and channel fabrication due to its flexibility. LBG electrodes were utilized and enhanced with $Ti_3C_2T_x$ MXene to improve electrical properties. The resulting cortisol sensor exhibited excellent performance, with a low detection limit of 3.88 pM and good selectivity. The study suggests that the developed $Ti_3C_2T_x$ MXene/LBG-based flexible

noninvasive patch could be extended for detecting other biomarkers or pathogens. Additionally, the integration potential with wearable electrochemical frontends for impedance signal monitoring and wireless data transfer for smartphone-based diagnostics is highlighted.

In [25], a label-free biosensor platform utilizing transition metal oxides for the detection of cortisol levels in saliva and sweat is presented, crucial for point-of-care testing (POCT). Through the immobilization of cortisol antibodies (ACAs) on a β-MnO2 CNs electrode via electrostatic interactions, an effective platform for cortisol detection is established. Bovine serum albumin (BSA) is employed as a blocking agent, minimizing non-specific adsorption on the electrode surface. Under optimized conditions, the ACAs/β-MnO2 nanostructured electrode demonstrates rapid and sensitive electrochemical impedance detection of cortisol, with a wide linear range (0.1 pM to 1500 pM) and a low limit of detection (LOD) of 0.023 pM. Notably, β-MnO2 CNs exhibit superior performance due to their large active surface area and efficient ACAs immobilization, enabling highly sensitive cortisol detection. Importantly, the biosensor shows excellent selectivity, with minimal interference from other biomolecules. Real sample analysis of human saliva and sweat further validates the assay's potential for clinical application, promising reliable cortisol detection in a point-of-care setting.

[26] presents a flexible biosensing platform utilizing conductive carbon fiber for monitoring sweat biomarkers, focusing on cortisol. By functionalizing the carbon yarn with ellipsoidal Fe_2O_3 and employing 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-Hydroxysuccinimide (NHS) chemistry, cortisol-specific antibodies are immobilized. Characterization techniques including XRD, FT-IR, Raman spectroscopy, and FE-SEM/EDS offer insights into structure and morphology[27]. The fabricated sensor exhibits excellent linearity ($r2 = 0.998$) over a range from 1 fg to 1 μg, with a sensitive detection limit of 0.005 fg/mL for cortisol. Additionally, investigations validate its robust performance, with cortisol detection in human sweat samples confirmed via a commercial chemiluminescence immunoassay (CLIA). Furthermore, a highly sensitive and conductive yarn-based flexible electrochemical sensor demonstrates broad sensing capabilities, exceptional selectivity to cortisol, and rapid response time, holding promise for commercialization in diverse applications, particularly with its potential integration into fabrics and garments.

In another study, a wearable, real-time, flexible microfluidic immunosensor was reviewed by [28] using a three-working-electrode LIG to detect cortisol, selected as the model stress hormone. The immunosensor was created by first performing the electrodeposition of 1H-pyrrole propionic acid through CV. Then, the anti-cortisol monoclonal antibody was covalently immobilized onto the surface using the EDC/Sulfo-NHS method. Following immobilization of the antibody, the surface was blocked with BSA. Subsequently, cortisol standards and HRP-cortisol were dropped onto the working electrode for 15 minutes, allowing competition between the labeled and circulating free cortisol in the sample for the free locations of the immunosensor. The LOD value of the immunosensor was 0.22 μM, and the cortisol sensor can respond within 1 minute.

To overcome limitations such as stress-inducing blood sampling and achieve continuous, non-invasive, real-time stress analysis at the molecular level, cortisol dynamics in human sweat are investigated using an integrated wireless sensing device by [29] Leveraging laser-induced graphene for electrochemical sensing, highly sensitive, selective, and efficient cortisol detection is enabled via a flexible sensor array. The first cortisol diurnal cycle and dynamic stress-response profile derived from human sweat are presented, highlighting exciting opportunities for non-invasive dynamic stress monitoring through wearable and portable sensing platforms. The potential of sweat hormone analysis using the integrated portable system, the GS4, is showcased, achieving sensitive and efficient stress hormone sensing with an assay time as low as 1 minute. The ability to extract crucial stress response and adaptation information from sweat cortisol is revealed by the study's findings, offering new avenues for fundamental psych neuroendocrinology studies and timely documentation of stress levels for day-to-day mental health monitoring. Despite focusing on physical stress stimuli, promising correlations with circulating hormones, diurnal cycles, and dynamic stress-response profiles are demonstrated by our integrated sensing approach, paving the way for personalized human performance and mental health management advancements.

In [30], The effectiveness of combining multi-layer graphene with pyrrole to develop an affordable and highly sensitive material for detecting cortisol is explored. Graphene nanoplatelets and pyrrole are dispersed in a solution containing 1M HNO_3 by sonication for 10 minutes, followed by centrifugation and polymerization through cyclic voltammetry, resulting in the creation of a graphene–pyrrole composite. Testing of this composite for detecting ultra-low levels of cortisol in artificial saliva, comparable to levels found in human salivary samples, reveals promising sensitivity. The composite's properties are analyzed utilizing Raman spectroscopy, while the interaction between the sensitive layer and cortisol is modeled using MarvinBeans software. Detection of cortisol levels as low as 0.5 ng/mL using cyclic voltammetry is demonstrated by this composite.

In summary, a sensor based on graphene and pyrrole for detecting cortisol levels in artificial saliva is investigated. The chosen materials enable environmentally friendly production and possess desirable qualities such as safety and affordability. After dispersing graphene and pyrrole nanostructures in a solution with 1M HNO_3 using ultrasound probe, followed by centrifugation and cyclic voltammetry for polymerization, the composite undergoes testing for various ultra-low cortisol concentrations in artificial saliva. Spectroscopic properties are examined using Raman spectroscopy, and the interaction with cortisol is modeled using MarvinBeans® software, providing insights consistent with experimental results.

Conformable, wearable biosensor-integrated systems are seen as a promising avenue for non-invasive and quantitative on-body detection of biomarkers in body fluids. However, the realization of such systems has been hindered by the difficulty in fabricating a soft affinity-based biosensor patch capable of precise on-body fluid handling with minimal wearer intervention and a simple measurement protocol. In [31], a conformable, wearable lab-on-a-patch (LOP) platform was demonstrated, comprising a stretchable, label-free, impedimetric biosensor and a stretchable

microfluidic device for on-body detection of the hormone biomarker, cortisol. The all-in-one, stretchable microfluidic device can precisely collect and deliver sweat for cortisol quantitation and offers one-touch operation of reagent delivery for simultaneous electrochemical signal generation and washing. High sensitivity required for detecting pM-levels of cortisol in sweat is enabled by three-dimensional nanostructuring of the Au working electrode. Sweat cortisol was quantitatively and accurately detected during exercise using the integrated LOP. This LOP is expected to open new horizons for non-invasive, highly sensitive, and quantitative on-body immunodetection for wearable personal diagnostics. Previous researchers had not successfully integrated sample-controlling microfluidics and a skin-mountable biosensor into fully functional lab-on-a-chip technology. Challenges were addressed using specifically designed valves and channels in the microfluidic device, enabling one-touch operation for simultaneous mediator delivery and washing. Through these techniques, an all-in-one, integrated microfluidics-electrochemical biosensor patch was constructed, providing wearable immunodetection of cortisol in human sweat. The proposed LOP platform, capable of completing all steps required to detect low-concentration biomarkers, shows promise, particularly if combined with a wearable electrochemical reader featuring circuitry for impedance signal readout and wireless data transmission. Additionally, wearable biosensor systems based on the LOP platform could potentially be extended to detect other biomarkers in sweat (such as cytokines and neuropeptides), therapeutic drugs in sweat, and a wider range of biomarkers in other biofluids, including interstitial fluids or wound exudate.

The development of wearable electrochemical sensors for monitoring various electrolytes and metabolites in raw sweat, saliva, or blood has been investigated extensively, showcasing advancements in sensor technology and addressing pertinent challenges in analytical chemistry and biomedical engineering [32], [33].

3. CONCLUSION

In this paper, we presented a review of recent studies concerning the detection of cortisol in sweat and saliva. Our review revealed that researchers have successfully developed various sensors for this purpose. However, a notable trade-off exists between the cost and sensitivity of these fabricated sensors. While some prioritize achieving lower detection levels, others concentrated on enhancing sensitivity or stability in electrochemical sensor performance. This review sheds light on the diverse strategies employed by researchers in tackling the challenges associated with cortisol detection, providing insights into the ongoing advancements and future directions in this field.

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