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Nucleic acid-based wearable and implantable electrochemical sensors

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The rapid advancements in nucleic acid-based electrochemical sensors for implantable and wearable applications have marked a significant leap forward in the domain of personal healthcare over the last decade. This technology promises to revolutionize personalized healthcare by facilitating the early diagnosis of diseases, monitoring of disease progression, and tailoring of individual treatment plans. This review navigates through the latest developments in this field, focusing on the strategies for nucleic acid sensing that enable real-time and continuous biomarker analysis directly in various biofluids, such as blood, interstitial fluid, sweat, and saliva. The review delves into various nucleic acid sensing strategies, emphasizing the innovative designs of biorecognition elements and signal transduction mechanisms that enable implantable and wearable applications. Special perspective is given to enhance nucleic acid-based sensor selectivity and sensitivity, which are crucial for the accurate detection of low-level biomarkers. The integration of such sensors into implantable and wearable platforms, including microneedle arrays and flexible electronic systems, actualizes their use in on-body devices for health monitoring. We also tackle the technical challenges encountered in the development of these sensors, such as ensuring long-term stability, managing the complexity of biofluid dynamics, and fulfilling the need for real-time, continuous, and reagentless detection. In conclusion, the review highlights the importance of these sensors in the future of medical engineering, offering insights into design considerations and future research directions to overcome existing limitations and fully realize the potential of nucleic acid-based electrochemical sensors for healthcare applications.

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1. Introduction

Biomarker information collected through blood sample analysis offers critical insights into health management. However, this traditional approach is intermittent, requiring frequent

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clinic appointments and invasive blood draws. The emerging fields of implantable and wearable biosensing technologies present a dynamic shift towards continuous, remote monitoring of health analytes. These innovations promise a new era of precision medicine by allowing the collection of longitudinal health data with biochemical sensors capable of measuring diverse biomolecular targets with high sensitivity and robust stability (Fig. 1).[1–9]

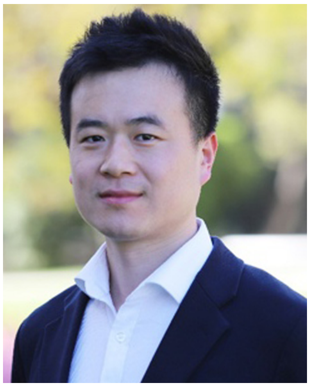
Implantable sensors may provide real-time insights into circulatory biomarkers in the blood and interstitial fluid (ISF), as well as localized indicators of tissue health and organ function. For instance, a biosensor placed in the brain can effectively monitor neurochemical signals associated with mood, cognition, and brain health.[10] Innovations in biofluid sampling and collection have enabled wearable non-invasive analysis of health biomarkers through alternative biofluids such as ISF, sweat, saliva, breath condensate, and tears.[11] With different secretory mechanisms and biomolecular partitioning, each of these fluids offers a unique lens on the body's internal state. Wearable microneedle arrays can be used to monitor circulatory biomarkers in ISF, while sweat may be collected from the skin surface and monitored for a wide range of small molecules such as amino acids, metabolites, hormones, ethanol, and therapeutic and abused drugs.[3,11,12]

The development of wearable and implantable biochemical sensors is considerably more challenging than creating activity trackers or traditional lab-based sensors. The complexity arises from the need for these devices to not only enable continuous on-body biochemical sensing but also ensure a reliable and precise analyte measurement in the complex biofluid matrix. This requires highly specific biorecognition elements with fast binding kinetics, highly efficient signal transduction, and *in situ* regeneration capabilities for repeated measurements.[13,14]

Conventional protein-based sensors relying on antibodies and enzymes are challenging to synthetically engineer for novel and diverse molecular targets. While burgeoning biotherapeutic research has enhanced synthetic antibody discovery and optimized high affinity target binding, a lack of label-free electrochemical signal transduction and sensor regeneration has limited their use in continuous on-body sensing applications. Naturally occurring redox enzymes, such as glucose oxidase, address these continuous sensing challenges, yet they have limited generalizability due to the difficulty of simultaneously engineering for selective binding of new targets and high catalytic activity.[15] Proteins thus present a gap in biosensing that easily modifiable and amplifiable nucleic acids can bridge.

With the advent of systematic evolution of ligands by exponential enrichment (SELEX), nucleic acid sequences have been designed to bind with high affinity to molecular targets beyond the antisense strand. Employing nucleic acids as biorecognition elements holds great significance in implantable and wearable sensor design as they possess robust binding capabilities, ensuring selective detection of specific molecules. The high affinity of nucleic acids also translates to minimal background noise, further enhancing the reliability of the measurements. Moreover, their ease of synthesis and ability to be regenerated make them both effective and economical choices for sensor design.[14,16,17]

This review discusses how nucleic acid-based sensors have expanded continuous electrochemical biosensing capabilities, especially in small molecule detection. Various nucleic acid



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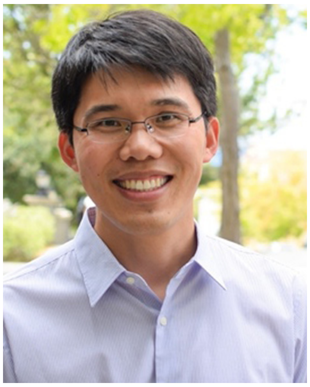

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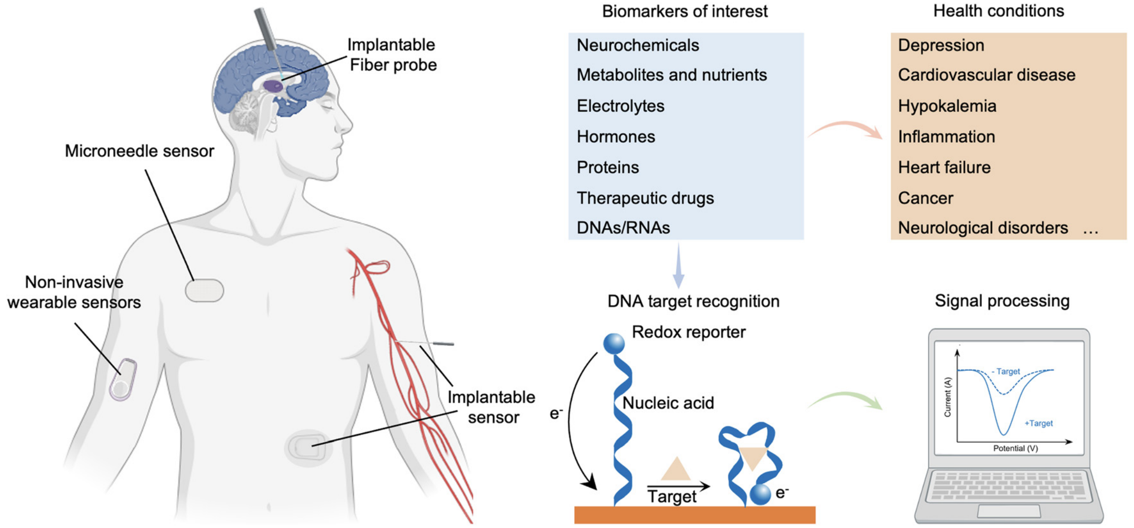

Fig. 1 Overview of nucleic acid-based implantable/wearable electrochemical sensors.

design schemes are presented for biorecognition and electrochemical signal transduction, including strand displacement and aptamer structure switching, as well as redox probe manipulation and transistor-based sensing, respectively. The role of nucleic acid sensors in continuous sensing is contextualized through key examples in wearable and implantable sensing platforms. Lastly, design criteria for the ideal continuous nucleic acid sensor are summarized with an outlook towards the next generation of wearable and implantable nucleic acid sensors.

2. Nucleic acid-based sensing strategies

Nucleic acid hybridization is one of the most specific biorecognition events in nature. Predictable base pairing and hydrogen bond interactions make nucleic acids easy to engineer as nucleic acid bioreceptors and malleable to non-nucleic acid target biorecognition. High-throughput nucleic acid amplification enables efficient, low-cost bioreceptor discovery. Well-studied, unique properties of deoxyribonucleic acid (DNA) allow for nucleic acid-specific sensor designs that might exploit DNA hybridization, thermal denaturing, and DNA's negatively charged phosphate backbone to name a few. For these reasons, nucleic acid-based sensors have emerged as versatile and significant tools for biochemical sensing.

In this section, we delve into the variety of nucleic acid biorecognition elements and discuss the signal transduction mechanisms employed in implantable and wearable sensor applications (Fig. 2). Our analysis encompasses the key categories of nucleic acid recognition elements, namely single-stranded nucleic acid probes and aptamers, which serve as both direct binding and strand-displacement sensing agents. Additionally, hybrid mechanisms that combine properties of nucleic acid strands with the biorecognition capability of proteins and antibodies are gaining prominence in the realm of innovative sensing approaches. Nucleic acid structure switches leveraging binding-induced conformational changes are dually explored as selective biorecognition elements and effective transducers. We then discuss signal transduction methods with a primary focus on redox probe-based electrochemical sensing and transistor-based sensing.

2.1 Biorecognition

Nucleic acid sensing of genetic markers, cell-free DNA, and pathogenic DNA relies on direct binding of complementary DNA (cDNA) probes. Assuming sufficient target DNA is present for interaction (possibly requiring amplification), spontaneous and selective hybridization will occur. Nucleic acid biorecognition was improved upon with the introduction of self-hybridizing hairpin molecular beacons that transduce the precise recognition of complementary targets to a detectable signal.[18] The molecular beacon sensing scheme has served as a springboard for modern nucleic acid-based sensor designs. With the advancement of reproducible, high-throughput nucleic acid synthesis, nucleic acid binding capabilities have expanded to non-nucleic acid targets. We describe the key nucleic acid-based biorecognition elements that have been realized for wearable and implantable biochemical sensing. Our discussion focuses on aptamers and hybrid mechanisms, such as clustered regularly interspaced short palindromic repeats (CRISPR)-Cas systems and protein-coupled molecular pendulums.

2.1.1 Aptamers. The capability of nucleic acids to bind small ligands motivated the *in vitro* selection of single-stranded ribonucleic acid (RNA) and DNA oligonucleotides that bind to specific ligands.[19] This new class of nucleic acid binding species termed aptamers could bind a wide variety of molecular targets for therapeutic and diagnostic applications. Aptamers noncovalently interact with ions, small molecules, peptides, proteins, and hormones.[20] Like molecular beacons, aptamers undergo binding-induced conformational changes

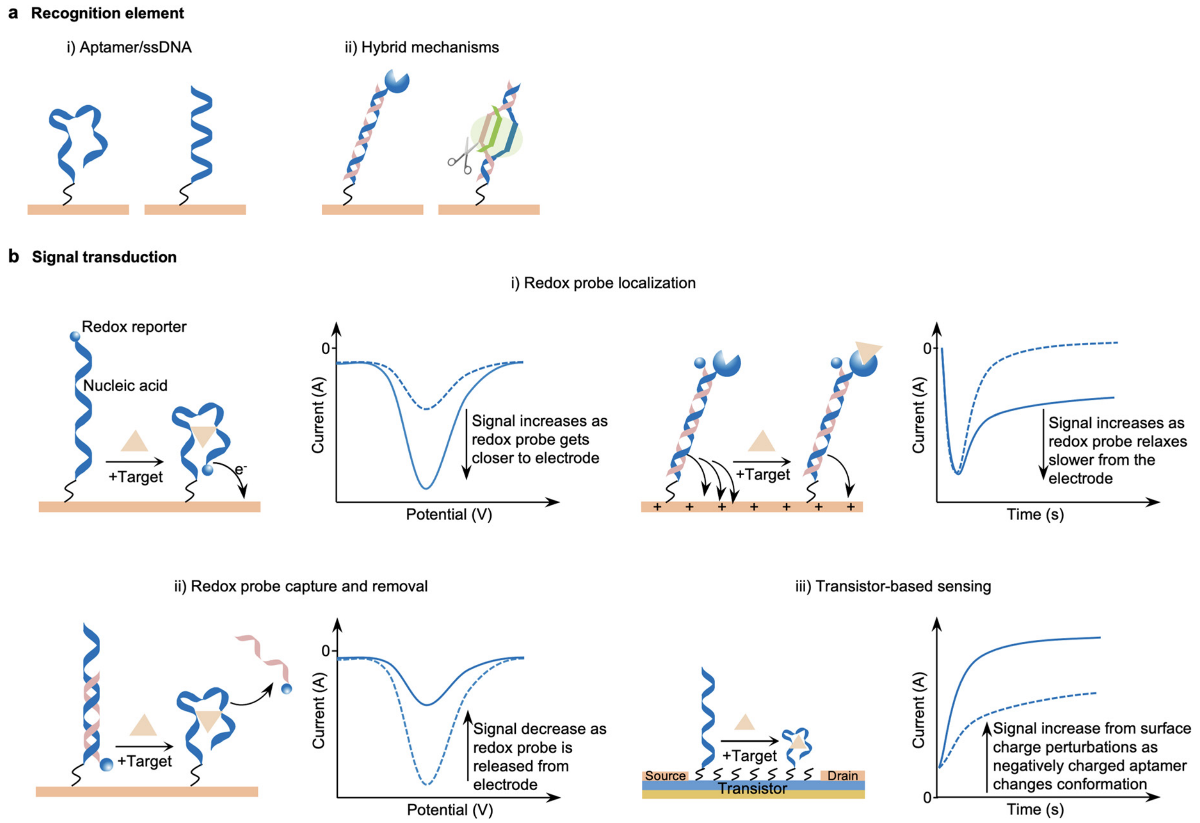

Fig. 2 Nucleic acid-based implantable/wearable sensing strategies: (a) classes of available nucleic acid-based recognition elements. (b) Signal transduction strategies, including redox probe localization, redox probe capture and removal, and transistor-based sensing.

based on their self-hybridized hairpin structures.[21] The structure-switching behavior allows aptamers to also transduce the biorecognition event to an optical or electrochemical signal.

An *in vitro* method known as SELEX is widely used for aptamer discovery and selection of structure-switching aptamers.[21–23] In this method, a library of randomized RNA or DNA recognition sequences undergoes a variety of successive selection rounds to tune the selectivity and affinity of the aptamer to the target. The binding kinetics and signaling response is evaluated for the final selected sequences. This iterative process is highly customizable with alternative library designs and innovative selection rounds having the potential to produce a variety of structure-switching aptamers.[21]

Aptamer structure plays a significant role in the signal transduction and sensor design scheme. Aptamers are 20–100 nucleotides in length yet can form a variety of structures including duplexes, hairpins, and G-quadruplexes that are either stabilized or destabilized through noncovalent target interactions.[20,24] Structure-switching classes of aptamers include stem-loop aptamers, split aptamers, strand-displacement aptamers, and triple-helix aptamers.[21,25] Stem-loop aptamers are based on the molecular beacon designs with a hairpin structure stabilized by a self-hybridized stem region of optimal length.[21] Split aptamer designs cleave a target-binding sequence at non-binding loop regions to create fragments that only associate in the presence of the target. This design may help to preserve structural elements key to target binding, but it also presents the challenge of coordinating the target-specific assembly of multiple components. Strand-displacement aptamers involve a complementary cDNA strand that is initially hybridized to the aptamer. Upon binding of the target molecule to the aptamer, the cDNA strand is displaced, signaling the presence of the target. The triple-helix molecular switch involves a hairpin aptamer that is stabilized by an oligonucleotide sandwiched between the stem strands.[25] Similar to strand-displacement aptamers, the target-binding of the outer hairpin results in dissociation from the sandwiched strand, which then participates in signal transduction. In any case, the aptamer design process requires thermodynamic optimization between the bound and unbound states that is adjusted for by the introduction of destabilizing mutations.[17,21] Aptamers thus face a recurring challenge of bioreceptor design: the trade-off between affinity and dissociation, key factors in determining a biosensor's sensitivity and regeneration capability for repeated use.

2.1.2 Hybrid mechanisms. Programmable CRISPR-Cas gene editing techniques have demonstrated highly selective recognition of target nucleic acid sequences that may be adapted for biosensing. The hybrid system relies on guide RNA (gRNA) hybridization to a target sequence to activate Cas enzyme cleavage of the target DNA.[26] CRISPR-Cas DNA sensing surpasses the detection accuracy of hybridization-based electrochemical DNA sensors enabling point-mutation analysis.

Electrochemical CRISPR (E-CRISPR) sensing is performed using an electrode functionalized with a redox-labelled cDNA strand which captures the target single stranded DNA (ssDNA). CRISPR-Cas9 and CRISPR-Cas12a systems are typically used to recognize the target sequence and cleave the cDNA strand releasing the redox probe from the electrode surface and producing a measurable change in the redox current.[26] E-CRISPR signal transduction has also been performed using a functionalized graphene field effect transistor.[27] E-CRISPR sensing enables ultrasensitive label-free detection of unamplified target genes and pathogenic DNA.[27–29] Combined aptamer-CRISPR systems allow for molecular detection beyond nucleic acids.[28] Due to their modular design and high accuracy, CRISPR-Cas sensors demonstrate great promise for wearable health monitoring.[30,31]

DNA can also be coupled with traditional bioreceptors, such as antibodies, to form a hybrid molecular switch. Antibody-based assays for small molecule sensing are often competitive assays with multi-step labeling. Linked DNA scaffolds can be used to attach a molecular competitor within the vicinity of the antibody enabling continuous concentration-dependent competitive binding and fluorescent beacon signaling. Antibody-switches have been used to continuously sense cortisol and the cardiac glycoside digoxin in human blood.[32,33] In a molecular pendulum design scheme, a DNA linker anchors the bioreceptor to the electrode surface and provides a means for signal transduction with a redox probe-labelled cDNA. This hybrid mechanism allows for high affinity target binding at picomolar levels and label-free signal transduction by exploiting the electrostatic properties of DNA. Molecular pendulum designs have been demonstrated using antibodies for a variety of targets including troponin I, B-type natriuretic peptide (BNP), interleukin-6, immunoglobulin E, and SARS-CoV-2 spike protein.[34,35] Molecular pendulum designs utilizing aptamer recognition elements have also been demonstrated for BNP and N-terminal pro BNP (NT-proBNP).[36] With a modular biorecognition element, hybrid molecular switches expand the sensing capabilities of nucleic acid-based sensors to targets beyond nucleic acids and small molecules.

2.2 Signal transduction

Nucleic acid sensing has traditionally relied on optical signal transduction methods. A fluorophore interacts with a quencher based on nucleic acid molecular interactions to produce a concentration-dependent fluorescent signal *via* fluorescence resonance energy transfer (FRET). Due to the simple signal transduction, molecular beacon fluorescence may be modulated in a variety of ways, including strand displacement, CRISPR-Cas cleavage, and aptamer structural switches. FRET quenching has played a significant role in aptamer beacon discovery enabling the screening and selection of aptamer sequences with significant conformational changes upon molecular binding.[21,22,37] However, for wearable and implantable applications, optical systems face challenges in miniaturization and form factor in comparison to electrical systems. Fortunately, these optical techniques are easily translated to electrochemical methods by using redox moieties like methylene blue (MB) and ferrocene (Fc).[38] Additionally, nucleic acid functionalized transistors act as both electrochemical transducers and amplifiers further enhancing molecular detection capabilities. Here, we provide an overview of how nucleic acid-based biorecognition mechanisms are employed for transducing biomolecular binding events into measurable electrical signals.

2.2.1 Redox probe localization. For electrochemical signal transduction, the nucleic acid detection probe is often labelled with a redox moiety like MB to observe target-dependent changes in electron transfer. The electron transfer of a redox reporter can be manipulated by changing the distance of the reporter to the working electrode surface. Electrochemical aptamer-based (EAB) sensors utilize a redox moiety-labelled aptamer attached to the electrode surface to measure the target binding-induced conformational change. The change in position of the redox probe can be interrogated using a variety of electrochemical techniques, including square wave voltammetry (SWV), cyclic voltammetry (CV), chronoamperometry (CA), intermittent pulse amperometry (IPA), and electrochemical impedance spectroscopy (EIS).[17,39]

Amperometric techniques directly measure the current decay rate from the surface-tethered redox probes. In the folded state, the redox reporter moves closer to the surface and experiences a stronger electric field for accelerated electron transfer measured by a faster current decay rate. The time constant of the measured current is easily optimized for by overlaying chronoamperometric traces.[39] CA and IPA allow for rapid time-resolved measurements limited only by data acquisition rates.[40] A tobramycin EAB sensor response was demonstrated as fast as 2 ms using IPA interrogation.[39]

SWV is the most common method for EAB sensor interrogation. SWV potential scans result in a peak current at the reporter's redox potential. This technique is favored for its measurement sensitivity by limiting the nonfaradaic current contribution. A key SWV parameter for EAB measurement is the SWV frequency, which relates to the optimized time constant used in amperometric methods.[41] At high frequencies, fast electron transfer of probes close to the electrode surface is captured. At low frequencies, slow electron transfer of probes far from the electrode surface is captured. Depending on the SWV measurement frequency and binding dynamics of the aptamer probe, the current will vary logarithmically with target concentration in either a signal-off or signal-on manner. The SWV amplitude also requires optimization since reduction and oxidation rates of the redox reporter vary differentially between the bound and unbound states.[41]

The intrinsic electron transfer of the chosen redox moiety as an additional sensor design factor plays a significant role in maximizing the EAB signal response. Anthraquinone and Fc redox reporters exhibit slow and fast electron transfer, respectively, relative to MB, limiting these responses to either the signal-on or signal-off regime.[41] In contrast, MB-coupled aptamers often have a signal-off, nonresponsive, and signal-on SWV frequency, enabling normalization calculations to account for signal drift and batch variation.[42,43]

For molecular pendulum signal transduction, the redox-labelled pendulum arm is manipulated using electrostatic attraction. When a positive potential is applied to the electrode, the negatively charged DNA linker bends toward the electrode surface. As the angle between the electrode and the pendulum arm decreases, the redox probe at the terminus of the cDNA strand moves closer to the electrode surface and down the electric field gradient. By choosing a redox reporter that is oxidized at a positive potential, such as Fc, electron transfer by the redox reporter can be measured as current. While the DNA linker arm experiences an electrostatic force towards the electrode surface, the terminal biorecognition element contributes significantly to an opposing hydrodynamic drag force. Binding of the target analyte to the molecular pendulum only increases the drag and subsequent transit time towards the electrode surface. The transit time is then correlated to the current decay such that current measurements at a chosen time constant from a chronoamperometric trace are correlated with the target concentration. This technique maintains strong performance across different biofluids, such as blood, saliva, sweat, tears, and urine, despite changes in viscosity impacting the molecular pendulum transit time.[34] Rather, the signal transduction is significantly influenced by the size of the target. A prime example of this is in the molecular pendulum's ability to distinguish between free spike protein and a SARS-CoV-2 viral particle using the same anti-spike antibody molecular pendulum.[35] The molecular pendulum is thus a promising nucleic acid-based approach for ultrasensitive, label-free sensing of peptides and proteins.

2.2.2 Redox probe capture and removal. Standard structure switching EAB sensors are limited in sensitivity by high background current and low transduction efficiency. The disadvantage of redox probe localization is that the signal response is low due to a non-zero background signal from redox probes maintained within proximity to the electrode surface. In contrast, redox probe capture and removal allows for clearly defined bound and unbound signal states, enhancing the signal response and dynamic range. This method is employed primarily by strand displacement switches and E-CRISPR. For strand displacement switches, the redox-labelled nucleic acid strand is not tethered to the electrode surface but is instead displaced during target-binding. As described previously, E-CRISPR sensors remove the redox probe by target-mediated Cas cleavage.

A strand-displacement aptamer sensor for estradiol achieved picomolar detection in sweat.[44] Target binding to an aptamer tethered at a biorecognition interface released the MB-conjugated cDNA, which was then captured by a tethered ssDNA at a parallel sensing electrode. This provides an indirect measure of the number of bound aptamer receptors. Since the aptamer is not the electrochemical probe, strand displacement is a promising signal transduction method for aptamer sequences with strong target binding affinity that may not elicit a large conformational change.

2.2.3 Transistor-based sensing. Biomolecular interactions may be amplified using transistor-based approaches for efficient signal transduction. The nonlinear signal response of transistors enables high sensitivity and a large dynamic range for target detection. In the case of field-effect transistor (FET) sensors, nucleic acid bioreceptors tethered to thin-film semiconductor channels alter the transconductance upon target binding resulting in significant changes in the measured source–drain current. Aptamer-based FET designs have been demonstrated for a variety of charged, neutral, and zwitterionic target biomarkers.[45–47] The structure switching aptamer redistributes surface charges as the negatively charged aptamer reorients closer to or away from the FET, thus changing the effective gate potential and source–drain current. Starting with a high transconductance is important to achieve a highly sensitive FET. Choosing a semiconductor with high electron mobility, such as indium oxide or graphene, enhances the intrinsic signal response. Additionally, interdigitating the source and drain electrodes increases the channel width, which increases the transconductance and the biorecognition area.[45] Although FETs have promising detection capabilities, fabrication challenges often lead to batch variation. Calibration methods may be used to help account for device-to-device variation.[46]

The Debye-length limitation requires that FET recognition events occur within the Debye length. A molecular electromechanical system (MolEMS) approach improved aptamer-FET sensing by using a rigid tetrahedral double-stranded DNA (dsDNA) base with a flexible ssDNA cantilever ending in an aptamer bioreceptor.[48] The aptamer in this setup has the flexibility to capture analytes beyond the Debye length, with an extension length of about 12 nm. Upon applying a negative gate potential, the cantilever was actuated towards the FET surface for FET detection within the Debye length. This technique demonstrated selective detection of proteins, small molecules, ions, and nucleic acids at sub-attomolar concentrations. Moreover, the tetrahedral base serves as an anti-fouling layer suppressing non-specific adsorption to the FET channel.

In contrast to FETs, organic electrochemical transistors (OECTs) are easily patterned using a conducting polymer channel interfacing with the gate electrode by a liquid electrolyte. Ease of fabrication allows OECTs to take on a variety of form factors to directly interface with biofluids for wearable and implantable sensing.[49–51] In this method, the binding of the target to the biorecognition element alters the gate current, which ultimately controls ion penetration into the volume of the polymer channel, typically made of poly(3,4-ethylenedioxythiophene) doped with poly(styrene sulfonate) (PEDOT:PSS). Changes in the ion-modulated channel conductance are then measured as the source–drain current. OECTs have been demonstrated to amplify the electron transfer kinetics of redox-labelled aptamers attached to the gate electrode.[52,53] Aptamer-based OECT transduction was optimized further by applying a pulsed square wave gate voltage sweep to better capture transient currents of the redox reporter.[51] Using gate and working electrodes of the same size, the OECT SWV signal amplifies the standard aptamer 3-electrode SWV signal by almost 200-fold. Also, relative OECT amplification increases as the channel size decreases, such that the signal is maintained in smaller devices. Pulsed aptamer-based OECT thus enables the miniaturization of EAB sensing.

To summarize, the modular design of nucleic acid-based sensors presents diverse biochemical sensing opportunities. The regeneration capabilities of some aptamers demonstrates their potential as continuous sensing bioreceptors.[17] Label-free signal transduction using redox probes or transistors allow for automatic detection and repeated interrogation. Additionally, nucleic acid-based signal transduction mechanisms employ few components alluding to low-cost and facile fabrication for mass manufacturing. These characteristics make nucleic acid-based sensors highly translatable to wearable and implantable applications for continuous health monitoring.

3. Nucleic acid-based wearable sensors

Wearable biosensors have emerged as a progressive tool in the health and medical fields, offering the ability to continuously monitor physiological states in real-time through minimally invasive or non-invasive methods.[2,12,54,55] These devices analyze biochemical markers present in alternative biofluids such as sweat, tears, saliva, and ISF, providing valuable health insights without the discomfort or complexity associated with traditional blood tests.[56] The adaptation of nucleic acid-based biosensor systems for use in wearable technologies presents innovative opportunities as well as unique design challenges.

Key considerations in the development of these wearable nucleic acid-based biosensors include the stability of the nucleic acid components in diverse biofluids, the need for robust and reliable signal transduction mechanisms that function effectively in the wearable format, and the integration of data processing and communication technologies for real-time analysis and feedback.[12] Additionally, it is crucial that biomarkers present at lower concentrations in alternative biofluids are met with highly sensitive biosensors.[57] Wearable biosensors must be designed with user comfort and convenience in mind, ensuring that they are lightweight, flexible, and capable of performing continuous monitoring without interfering with daily activities. Addressing these challenges requires innovative materials science, bioengineering, and electronic design strategies to create wearable nucleic acid-based biosensors that are not only effective but also practical for everyday use. As we navigate these challenges, the potential for wearable biosensors to transform health monitoring and personalized medicine becomes increasingly tangible, promising a future where detailed physiological insights are seamlessly integrated into daily life.

3.1 Nucleic acid-based wearable microneedle sensors in ISF

Unlike subcutaneous implants, wearable microneedle patches offer a minimally invasive, painless method for the collection of ISF from the skin for post-sampling analysis or *in situ* real-time biosensing (Fig. 3a).[58,59] This innovative approach facilitates disease diagnosis and the monitoring of long-term drug pharmacokinetics through wearable devices.

The first use of microneedle EAB sensors for continuous molecular measurements was demonstrated in flow systems *in vitro* using single and multiplexed microneedle array configurations (Fig. 3b).[60] Recent EAB microneedle implementations have fallen short *in vivo* sensing performance despite using complex and expensive microneedle fabrication methods. A robust gold nanoparticle (AuNP) coated microneedle coupled with an EAB biosensing patch (μNEAB-patch) was introduced for minimally invasive, continuous, and real-time monitoring of drug pharmacokinetics in ISF (Fig. 3c).[61] Widely available clinical-grade needles optimized for dermal application are readily transformed with a single Au deposition step and aptamer coating to cost-effectively produce a high-quality working electrode for transdermal sensing. This simultaneously leverages the needle's sharpness for skin penetration and its conductivity for signal transduction. This approach enables the detection of a broad range of ISF analytes, particularly focusing on antibiotics with narrow therapeutic windows, demonstrating its potential for clinical applications in drug exposure monitoring through *in vivo* animal studies.[63,64]

Apart from the small molecule analysis from ISF, a wearable device utilizing *Natronobacterium gregoryi* Argonaute (NgAgo) coupled with tetrahedral DNA nanostructures was demonstrated for the sensitive and long-term monitoring of unamplified nucleic acids *in vivo*, targeting applications like sepsis detection through a microneedle biosensor integrated with a flexible circuit and a stretchable epidermis patch (Fig. 3d).[30,62] Specifically, the *in vivo* experiments, exploring the NgAgo/guide DNA recognition mechanism and signal processing, demonstrate the device's capability for real-time monitoring of cell-free DNA and RNA with femtomolar sensitivity over 14 days, offering a new approach for on-body nucleic acid detection and personal health management.

Microneedle based wearable sensors represent a pivotal innovation in the field of minimally invasive biosensing, enabling the real-time monitoring of ISF biomarkers for disease diagnosis and drug monitoring.[63] Despite their significant advantages over subcutaneous implants, including painless application and the ability to conduct post-sampling analysis or *in situ* biosensing, these devices face several operational challenges that can impact their performance and reliability. One such challenge is the poor signal-to-noise ratio (SNR) encountered in some EAB microneedle sensors, which can obscure the detection of target analytes and reduce the accuracy of the measurements. Additionally, the degradation of the sensing layer during *in vivo* operation presents a critical hurdle, affecting the sensor's longevity and functionality. These issues are compounded by the complexity and cost of the fabrication processes required to construct these advanced biosensing platforms, posing barriers to widespread adoption and clinical integration.

Recent advancements in microneedle technology indeed offer solutions to some of these challenges by enhancing the stability and conductivity of the microneedles, thus facilitating stronger aptamer immobilization for accurate biomarker detection *in vivo*. However, ensuring consistent and reliable skin

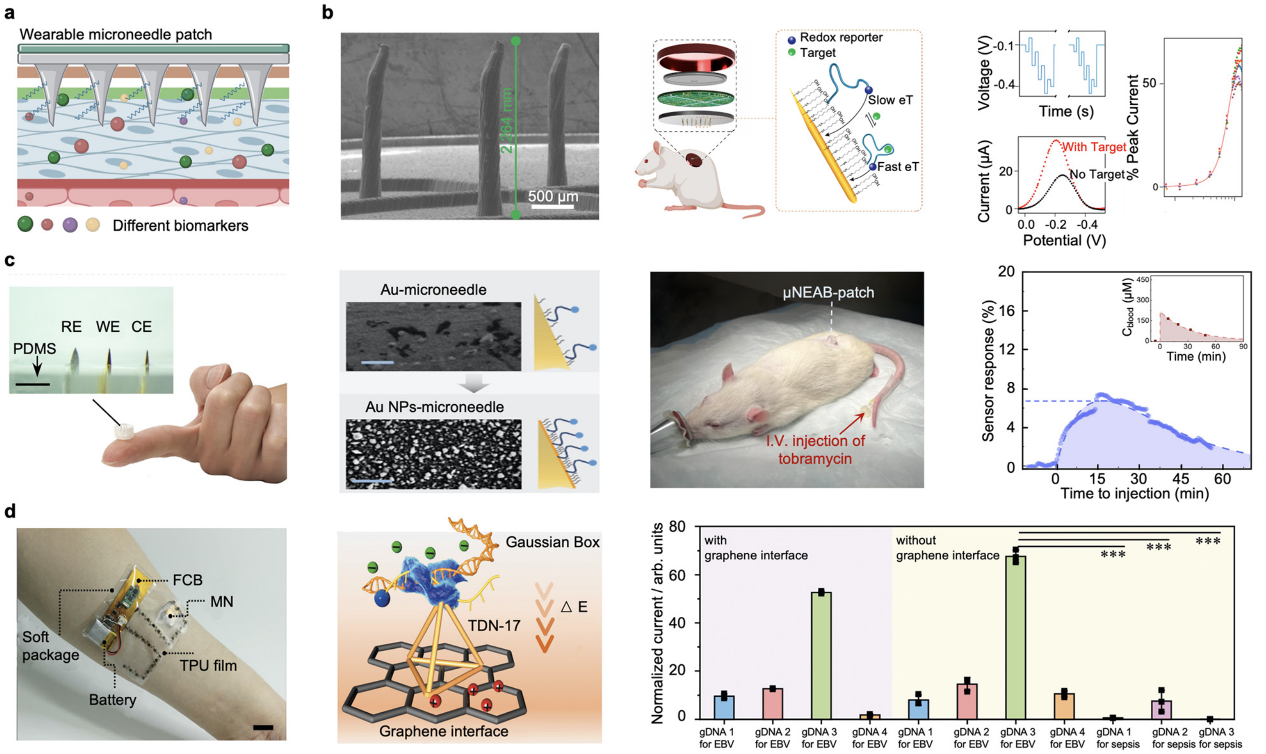

Fig. 3 Nucleic acid-based wearable microneedle sensor in interstitial fluid. (a) Principle of wearable microneedle sensor. (b) Microneedle aptamer-based sensors for continuous, real-time therapeutic drug monitoring. Reproduced with permission from ref. 60. Copyright 2022 Analytical chemistry. (c) Wearable microneedle-based electrochemical aptamer biosensing of various drugs with narrow therapeutic windows. Reproduced with permission from ref. 61. Copyright 2022 Science Advances. (d) Tetrahedral nanostructure-based *Natronobacterium gregoryi* Argonaute (NgAgo) for long-term stable monitoring of ultratrace unamplified nucleic acids. Reproduced with permission from ref. 62. Copyright 2024 Nature Communications.

penetration without causing tissue damage, alongside maintaining the sensor's integrity and performance over extended periods, remains a significant concern. Moreover, expanding the range of detectable analytes, particularly for complex biomarkers like unamplified nucleic acids, introduces additional complexities in sensor design and signal processing. Nonetheless, advancing microneedle-based wearable sensors towards clinical application necessitates overcoming these operational challenges through continued innovation in materials science, sensor technology, and fabrication techniques, ensuring that these promising devices can fulfill their potential in personalized healthcare and disease management.

3.2 Nucleic acid-based wearable sensors for non-invasive molecular analysis

Since extracting diagnostically useful ISF at sufficient volumes is challenging, non-invasive wearable devices provide an alternative pathway to clinical diagnostics (Fig. 4a).[12,65] In contrast to minimally invasive wearable microneedle-based devices, non-invasive wearable chemical sensors using alternative body fluids, such as sweat, saliva, and tears, can provide real-time analysis that is difficult to achieve in typical laboratory blood analysis.[1,11,12,66,67] Due to their diverse biomarker compatibility and a modular design scheme, the nucleic acid-based wearable devices can be designed to perform multiplexed measurements of biochemical markers in real-time that would be transformative for diagnostics. These low-cost devices enable remote, at-home health monitoring with high-resolution and time-resolved electrochemical recordings informing physiological and psychological dynamics.

Accessible secretory fluids such as sweat, saliva, and tears are highly filtered from their blood and ISF origins, leading to substantially lower biomarker concentrations than in blood. Towards this end, a flexible biosensor array utilizing a cortisol aptamer integrated with thin-film In_2O_3 FETs was reported for picomolar level cortisol detection (Fig. 4b).[46] The study tracked salivary cortisol levels in stress test participants and correlated these with diurnal sweat samples, leading to the development of an aptamer-FET smartwatch for continuous, real-time monitoring of cortisol. To meet the high demand for wearables mounted on the skin or integrated into clothing, the ultralightweight conductive nanofibers were electrospun to fabricate a liquid-ion gated FET on polyester for wearable cortisol aptamer sensing.[71]

Higher sensitivity and dynamic range may also be obtained by reducing the background current and improving the SNR of the sensor. EAB sensors typically rely on a conformation switch resulting in high background current due to the unclear position of the signal molecule in its unbound state. To improve upon this, a pseudoknot-assisted structure switching aptamer was implemented in a flexible electronic and soft microfluidic wearable platform for pH-calibrated, continuous, non-invasive

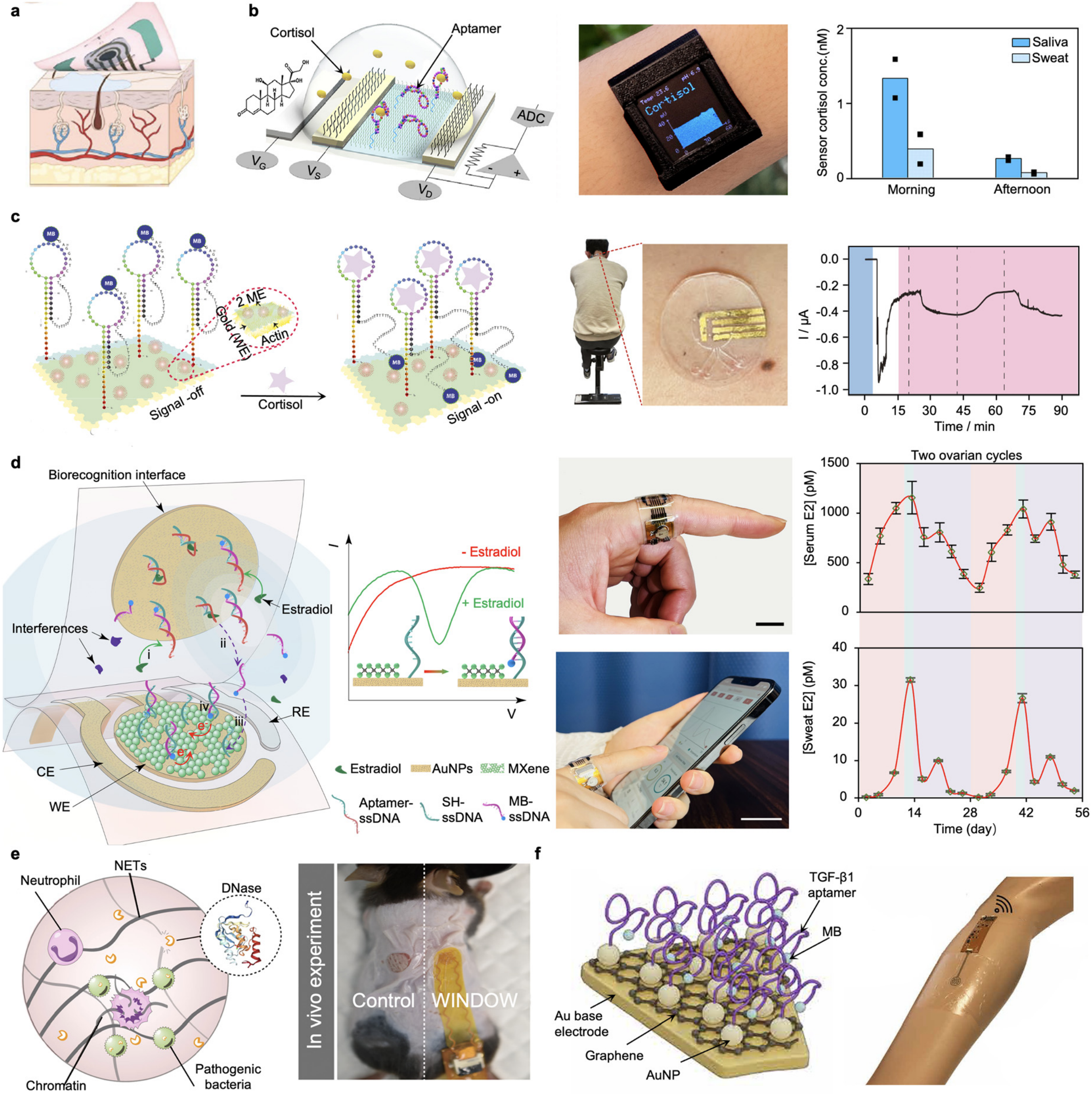

Fig. 4 Nucleic acid-based wearable sensors for non-invasive molecular analysis. (a) Principle of non-invasive wearable sensor. (b) Wearable aptamer-field-effect transistor sensor for non-invasive cortisol monitoring. Reproduced with permission from ref. 46. Copyright 2022 Science Advances. (c) A non-invasive pseudoknot-assisted aptamer wearable stress patch for real-time cortisol measurement. Reproduced with permission from ref. 68. Copyright 2023 Biosensors and Bioelectronics. (d) Wearable aptamer nanobiosensor for non-invasive female hormone monitoring. Reproduced with permission from ref. 44. Copyright 2024 Nature Nanotechnology. (e) A bacteria-responsive DNA hydrogel-based battery-free wearable sensor for wound infection monitoring. Reproduced with permission from ref. 69. Copyright 2021 Science Advances. (f) A multiplexed aptamer sensor array for *in situ* wound monitoring of inflammatory mediators and microbial proliferation. Reproduced with permission from ref. 70. Copyright 2021 Science Advances.

cortisol monitoring in sweat (Fig. 4c).[68] The pseudoknot tethers the redox reporter to the loop region of the unbound aptamer receptor, locking it in a distanced position from the electrode to minimize the background current. To address ultralow detection limits, unique redox probe capture and removal methods can be employed. A skin-interfaced wearable aptamer sensing platform was constructed using a target-induced strand displacement method, enabling non-invasive, *in situ* monitoring of female hormone levels in sweat (Fig. 4d).[44] The sensing scheme is amplification-free and reagentless, and yet achieves a picomolar detection limit using a signal-on approach coupled with a AuNP-MXene modified working electrode. This comprehensive system can realize sweat analysis at rest autonomously *via* iontophoresis induction and microfluidic sample collection. Additionally, to support sweat matrix variability, the sensing platform conducts real-time calibration utilizing a multivariable analysis of pH, ionic strength, and temperature. The platform then wirelessly sends measured and processed sweat hormone data to a mobile device application for review. Such skin-conforming microfluidic nucleic acid-based sensors have great potential to help individuals monitor their health non-invasively at-home.

Wound infections represent a considerable clinical challenge, necessitating timely detection for effective intervention. Wireless wearable sensing technology enables direct wound interfacing for advanced, home-based detection and management of medical conditions.[72–74] A bacteria-responsive DNA hydrogel was used to fabricate the battery-free wearable sensor for wound infection monitoring on the basis of pathogenic nuclease activity (Fig. 4e).[69] The sensor patch identifies clinical levels of *Staphylococcus aureus* before any visible signs of infection become apparent. Monitoring additional parameters in the wound bed can yield informed, proactive treatment for improved wound management. However, existing sensing technologies can only track a limited number of wound parameters. A flexible and multiplexed biosensing platform was developed by integrating an aptameric sensor array for measuring inflammatory mediators and physicochemical parameters (Fig. 4f).[70] This technology was demonstrated to enable *in situ* multiplexed wound monitoring in a mouse model and patients with venous leg ulcers. Its immense potential lies in personalizing wound management, thereby enhancing the healing outcomes of chronic wounds.

The advent of wearable biosensors for non-invasive monitoring of biomarkers in alternative biofluids, such as sweat, saliva, tears, and wound exudate, is a leap in diagnostics with transformative implications for personalized, at-home health monitoring. These needle-free devices offer a less intrusive alternative to traditional blood tests and ISF microneedles and enable the continuous tracking of physiological and psychological states in real-time. However, the operational challenges associated with working in alternative secretory fluids are significant and multifaceted, particularly when it comes to maintaining accuracy and reliability. Strain, temperature, pH, biofouling, and immune reactions under long-term use are critical factors that can affect sensor performance.[1–7,16,31,59,75] For example, the mechanical strain from daily movements can alter sensor readings, while temperature variations can affect the biochemical reactions that sensors rely on for biomarker detection. The pH of biofluids can also vary between individuals and over time, potentially impacting biosensor function. In the case of wearable technology for chronic wound management, significant challenges remain in their practical application. A smart dressing should maintain a moist environment to protect against secondary infections and promote tissue regeneration, while also absorbing and removing exudate from the wound.

Addressing these challenges requires innovative sensor design and materials science approaches to improve the resilience and adaptability of wearable biosensors. For instance, employing flexible, skin-like conductive materials that can conform to the wearer's movements and resist biofouling, alongside integrating microfluidic systems for sweat analysis that are calibrated for pH and temperature, can enhance sensor accuracy. For chronic non-healing wounds, a highly breathable, wearable smart dressing would shield the wound bed from bacterial infiltration and monitor for infection risk for early intervention. Furthermore, the development of signal-on detection mechanisms minimizing background current and boosting sensitivity showcases the importance of engineering solutions to biochemical challenges in wearable sensor design. As research and development in wearable biosensors advance, overcoming the operational challenges will enable these technologies to fully realize their potential, ushering in a new era of healthcare monitoring and personalized medicine.

4. Nucleic acid-based implantable sensors

Implantable medical devices are pivotal in modern healthcare, providing essential diagnostic, therapeutic, and regenerative functions across various clinical applications such as drug delivery, vital signs monitoring, and tissue repair.[76–78] Among these, nucleic acid-based implantable sensors stand out for their ability to offer real-time, reagent-free sensing of biomarkers through affinity-based molecular detection. These devices are designed with biocompatible housings, detection systems employing electrochemical or optical methods, and advanced communication interfaces for seamless data transmission.[79,80] Material selection plays a critical role in the development of implantable sensors, particularly when leveraging affinity probes such as nucleic acid-based recognition elements for the selective detection of neurochemical markers and circulating biomarkers.[81,82] The choice of materials is guided by the need for biocompatibility, ensuring that the sensors not only integrate seamlessly into the body's physiological environment without eliciting significant inflammatory responses but also maintain their functional integrity over time. Biocompatible materials, including polymers, metals, silicon, and their composites, are engineered to mimic the mechanical properties of native tissues and be biochemically inert to prevent an immune response and fouling, all towards facilitating accurate and stable sensing capabilities.[83] This engineering approach enables the deployment of implantable sensors that can monitor a variety of molecular targets directly within the cerebrospinal fluid (CSF) or through intravenous and subcutaneous routes for the analysis of biomarkers in blood and ISF.[11,80] By integrating nucleic acid-based recognition elements, these sensors offer the selectivity needed for the detection of specific neurotransmitters and circulating biomarkers, enhancing our ability to diagnose and monitor neurological conditions and systemic diseases.

4.1 Nucleic acid-based implantable neurochemical sensing

Nucleic acid-based implantable neurochemical sensors represent a critical approach in neurological monitoring and nervous system signaling. Utilizing the unique properties of nucleic acids, particularly aptamers, these sensors offer a highly selective and sensitive method for detecting and quantifying neurochemicals in

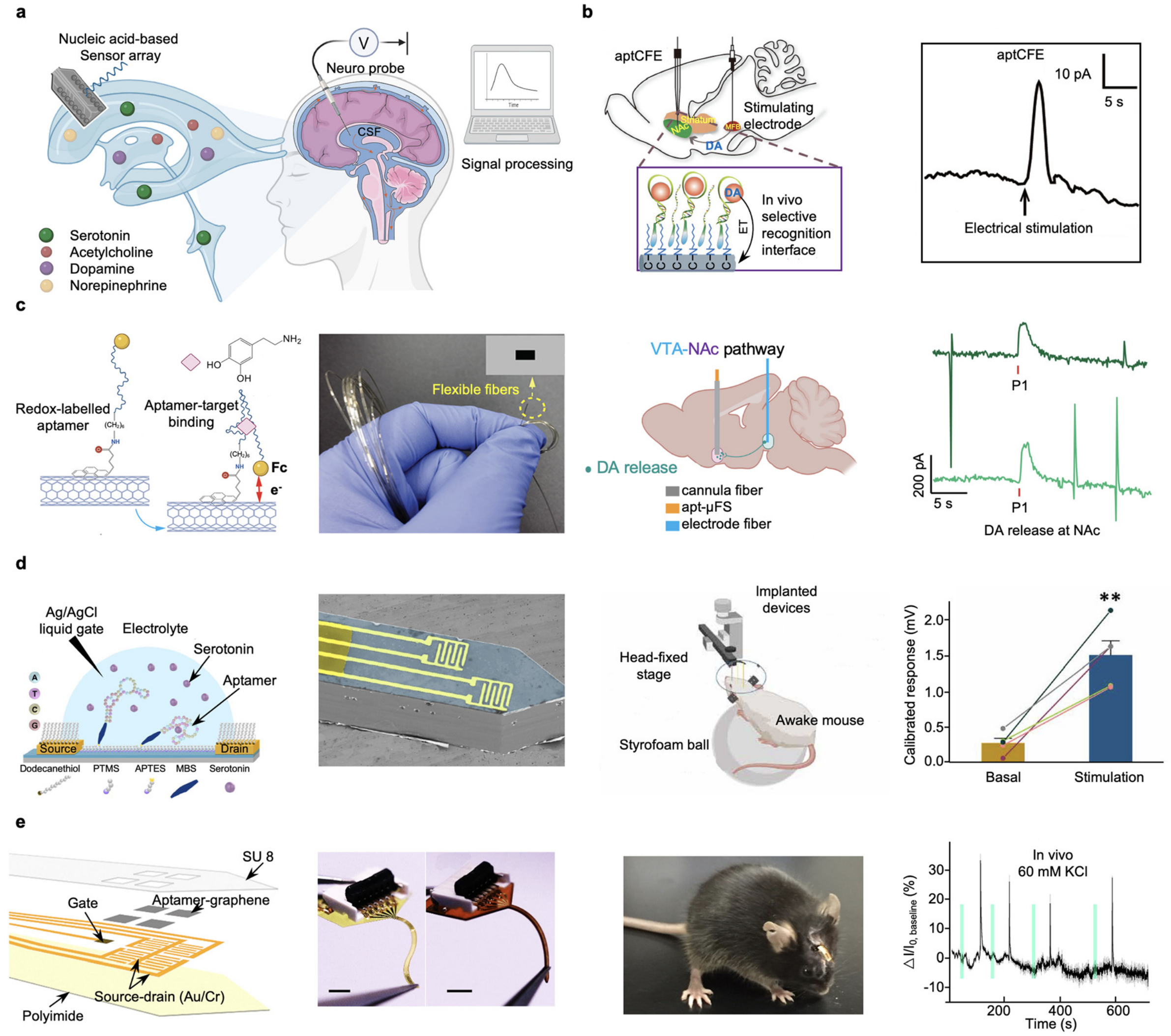

Fig. 5 Nucleic acid-based implantable neurochemical sensing. (a) Principle of implantable neurochemical sensing. (b) Aptamer cholesterol amphiphiles on the alkyl chain-functionalized carbon fiber microelectrodes for probing neurochemical dynamics. Reproduced with permission from ref. 87. Copyright 2020 Angewandte Chemie International Edition. (c) Aptamer-coupled microelectrode fiber sensor for *in vivo* dopamine detection. Reproduced with permission from ref. 88 Copyright 2023 Analytical chemistry. (d) Flexible and implantable neuroprobes with aptamer-field-effect transistor biosensors for neurotransmitter monitoring. Reproduced with permission from ref. 89. Copyright 2021 Science Advances. (e) Soft implantable aptamer-graphene microtransistor probe for real-time monitoring of neurochemical release. Reproduced with permission from ref. 90. Copyright 2022 Nano Letters.

real-time (Fig. 5a).[84] The levels of neurochemicals in the extracellular fluid are crucial for studying brain functions. However, monitoring these levels is challenging due to the chemical and physiological complexity of the central nervous system (CNS).[10] A variety of synthetic aptamers have been designed to bind neurotransmitters and other neurologically relevant molecules, making them ideal for continuous sensing applications.[85] This provides an invaluable tool for the continuous monitoring of neurochemical biomarkers, essential for understanding normal neural function, diagnosing neurological disorders, and evaluating the efficacy of treatments. For instance, monitoring fluctuations in neurotransmitters such as dopamine and serotonin provides insights into various psychiatric and neurodegenerative conditions, including depression, schizophrenia, Parkinson's, and Alzheimer's disease.[86]

The conventional use of rigid materials in implantable electrodes can induce adverse tissue responses that compromise sensor functionality. Traditional implantable electrodes, such as early neuroprobes, are made from rigid materials like silicon or metal, which significantly differ in stiffness compared to biological tissues.[85] For instance, silicon's stiffness is around 200 GPa, which is in stark contrast with the brain tissue's softness, measured at roughly 10 kPa.[91] This vast discrepancy in Young's modulus triggers immune reactions and the formation of glial scars, greatly impacting the biosensor performance. The immune reactions can cause signal degradation due to biofouling, while scar tissue formation can reduce the precision and sensitivity of the device over time by obstructing the interaction between the sensor surface and the surrounding tissue.[92] However, recent advances in soft and flexible electronics offer promising solutions to these challenges, suggesting a way forward for improving implantable sensor performance.

A strategy for interfacing aptamers with carbon fiber microelectrodes (CFEs) was developed to create highly selective systems for dopamine (DA) sensing *in vivo* (Fig. 5b).[87] An interfacial functionalization strategy was applied to assemble aptamer cholesterol amphiphiles (aptCAs) onto alkyl chain-functionalized CFE surfaces through noncovalent interactions, enabling aptamer immobilization on the CFE surface and neurotransmitter recognition with high sensitivity. The integration of high affinity aptamers into these platforms marks a significant advancement by addressing the issue of non-specific binding that hampers clinical translation. A flexible and multifunctional polymer-based fiber microelectrode was modified with Fc-tagged aptamers for highly selective DA detection, where the redox reaction of Fc serves as an indicator and signal output based on the target binding induced aptamer conformation change (Fig. 5c).[88] The study demonstrated the potential of fiber-based toolsets for multimodal exploration of brain pathophysiology, showing how these sensors can provide valuable insights into the neurochemical basis of neurological conditions.

Implantable aptamer-functionalized In_2O_3 FET neuroprobes were fabricated by high-throughput microelectromechanical system (MEMS) technologies (Fig. 5d).[89] Thiol-terminated aptamers designed for specific target recognition are attached to semiconductor surfaces of FETs, where conformational changes upon target capture lead to surface charge redistribution. This change can be detected by the voltage-gated semiconductor, thus aptamer-FET neuroprobes enable femtomolar serotonin measurement in brain tissue with minimal biofouling. Despite the necessity for flexible devices for neurotransmitter monitoring, the Young's moduli of many flexible substrates (such as PET) remain significantly higher than that of brain tissue. A viable approach is to make these materials thinner, since the bending stiffness is proportional to the cube of thickness following a rectangular beam geometry. Polyimide devices with thicknesses $<10\ \mu m$ have been shown to have optimal bending stiffness and conformal contact with tissue. The neuroprobe, incorporating an aptamer FET, was employed to develop a flexible and implantable sensor for monitoring DA, one of the small neurotransmitter molecules (Fig. 5e).[90] Aptamer configuration switching occurred *via* the recognition between aptamer and target DA, resulting in the augment of source–drain current due to p-doping effect on the graphene channel. Compared with In_2O_3-based FET neuroprobes, a graphene-based microtransistor is a three-terminal electronic device because graphene can act as a channel material between the source and drain terminals. As graphene offers much higher charge mobility compared with In_2O_3, graphene-based FET neuroprobes are capable of good sensing performance. Graphene also has good biocompatibility and chemical inertness ideal for *in vivo* applications. An implantable sensing platform was established for real-time serotonin monitoring using a aptamer-graphene microtransistor probe that can capture DA release *in vivo* selectively and sensitively.[82,93]

Overall, nucleic acid-based implantable sensors for neurochemical detection mark a significant advancement in neuroscience, offering a path to real-time monitoring of critical neurotransmitters like dopamine and serotonin. Despite their high selectivity and sensitivity enabled by aptamer technology, these sensors face operational challenges such as the mismatch in mechanical properties between traditional rigid sensor materials and soft brain tissue, which can trigger immune responses and scar tissue formation, impairing sensor functionality over time. Recent developments in flexible and soft electronics are addressing these challenges, with innovations such as carbon fiber microelectrodes and polymer-based fibers that are more compatible with brain tissue. However, ensuring stable nucleic acid immobilization on these flexible substrates remains a hurdle. Addressing these operational challenges will help drive the clinical translation of nucleic acid-based implantable sensors interfacing with the brain and other organs throughout the body.

4.2 Nucleic acid-based implantable sensing of circulating biomarkers

Circulating biomarkers in the blood and ISF are of paramount importance for personalized healthcare. These biomarkers including drugs, metabolites, hormones, and proteins offer a window into the body's physiological and pathological states.[94–97] Their detection and quantification can provide critical insights into an individual's health status, enabling early diagnosis of diseases, monitoring of disease progression, and evaluation of treatment responses (Fig. 6a). Circulating biomarkers in the blood can indicate a wide range of conditions, from cardiovascular diseases and cancer to inflammatory and neurodegenerative disorders. In addition to circulating biomarkers that filter from blood into ISF, localized ISF biomarkers can reveal surrounding tissue health and metabolic processes. The ability to accurately monitor these biomarkers present vast possibilities for preventive medicine, tailored treatment plans, and real-time tracking of health outcomes, making it a cornerstone of personalized medicine.[14,98,99]

Real-time therapeutic drug monitoring is pivotal for personalized dosing, tailoring treatment to the unique metabolic profiles of individual patients. Regrettably, there is only a limited number of targets that can be continuously measured in real-time, *i.e.* oxygen, lactose, glucose. Addressing this limitation, the microfluidic electrochemical detector for *in vivo* continuous monitoring (MEDIC) was introduced.[101] This device's modularity allows for the reagentless monitoring of a diverse array of molecules by simply swapping out aptamers, thus broadening the applications for continuous drug monitoring. Intravenous EAB sensors were evaluated for the continuous, real-time monitoring of multiple circulating drugs, including aminoglycoside antibiotics and the chemotherapeutic doxorubicin, in a rat model (Fig. 6b).[100] The EAB sensor scheme was further employed for vancomycin, a drug with a narrow therapeutic window challenged by individual metabolic variations, where they successfully demonstrated feedback-controlled drug delivery.[102] In parallel, to focus on the tumor

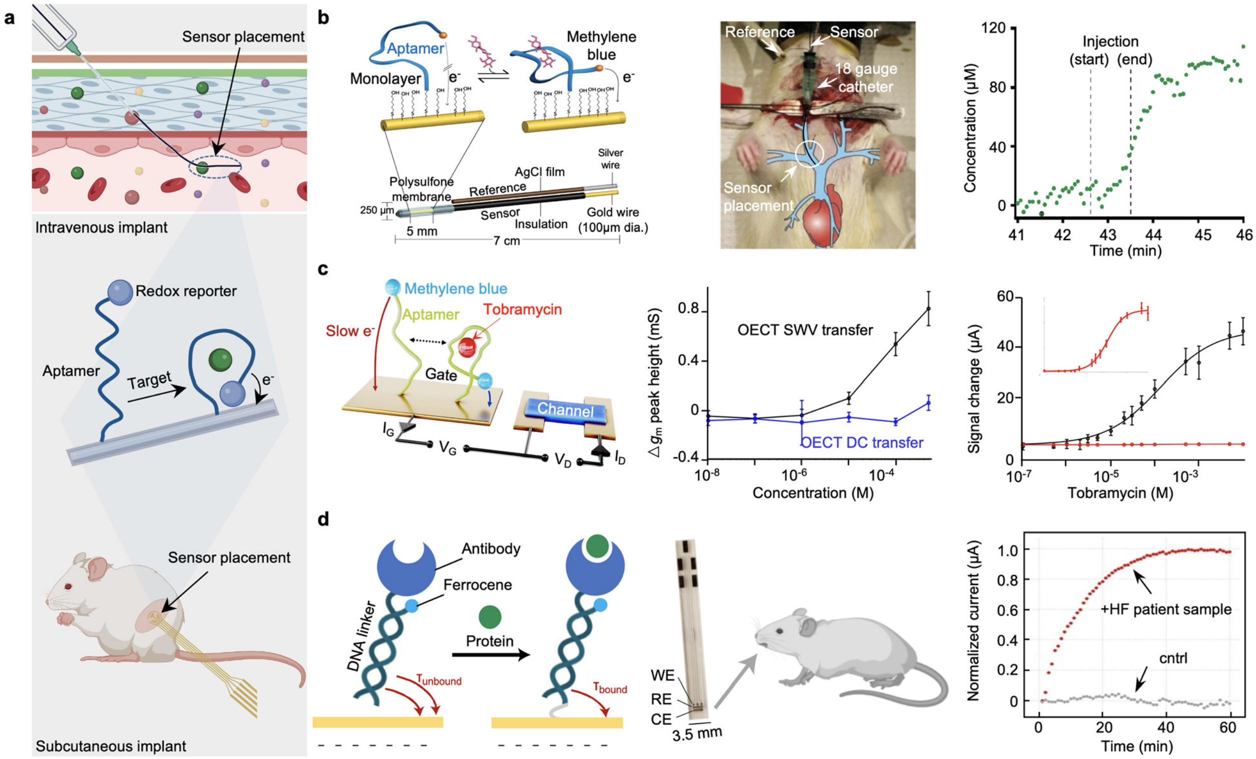

Fig. 6 Nucleic acid-based implantable sensing of circulating biomarkers. (a) Principle of implantable sensing of circulating biomarkers. (b) Electrochemical aptamer-based sensors for real-time measurement of therapeutic drugs in the bloodstream. Reproduced with permission from ref. 100. Copyright 2017 Proceedings of the National Academy of Sciences. (c) Square wave gate potential profiles support high-gain aptamer-based organic electrochemical transistor sensing. Reproduced with permission from ref. 51. Copyright 2022 Science Advances. (d) Reagentless biomolecular analysis using a molecular pendulum. Reproduced with permission from ref. 34 and 36. Copyright 2023 Angewandte Chemie. Copyright 2021 Nature Chemistry.

microenvironment, a microelectrode sensor array was utilized to directly measure drug levels within tumor sites in rodents.[103] Additionally, to address the fouling related signal drift, biocompatible agarose gel was utilized as a protective layer to fabricate the gel-protective implantable EAB sensor for *in vivo* real-time drug analysis.[104] This innovative approach offers critical insights into drug distribution challenges within tumors, such as irregular blood flow and cellular density, potentially influencing the efficacy of cancer treatments.

As discussed previously, nucleic acid-based transistor designs provide simultaneous signal transduction and amplification, a notable advancement over conventional electrode-based sensors. An aptamer-based OECT applied a square wave potential across its aptamer-decorated gate electrode to achieve a level of current modulation in the transistor channel surpassing that of traditional transistor interrogation (Fig. 6c).[51] The OECT design demonstrated signal amplification in whole blood and upon miniaturization, enabling potential future application in implantable sensors. The authors postulate that signal amplification by OECT may also extend the operation life of the sensor since signal degradation of nucleic acid-based sensors remain a challenge for the field. Beyond circulatory measurements, miniaturized OECT arrays may be the future of biochemical sensing for high resolution spatial measurements at the brain and muscle tissue interface.

Building on these technological strides, the quest for creating sensors capable of identifying molecular analytes in biological fluids without external reagents have opened new frontiers in personalized health monitoring. However, the diversity of molecular targets detectable by such reagentless sensors has been limited until now. Addressing this, a reagentless sensing technique was introduced that leverages the movement of a molecular structure, reminiscent of an inverted pendulum, whose motion is influenced by an electric field in response to the binding of an analyte (Fig. 6d).[34,36] This technique assesses the sensor's movement by analyzing the electron transfer rates to an indicator molecule, utilizing time-sensitive electrochemical analysis to monitor the sensor's unique motion. This allows for the continuous, real-time detection of a wide range of analytes, demonstrating compatibility with various biological fluids such as blood, saliva, urine, tears, and sweat, thereby enabling data collection directly within living organisms. Blood has been a primary biofluid for sampling in the lab and at-home and is the standard biofluid for biosensor *in vitro* validation and testing. Synthetic nucleic acid-based bioreceptors can be tuned to be highly selective for their target, and membrane coatings and surface chemistry can also be used to prevent non-specific binding and adsorption. Measurements in filtered biofluids such as ISF and sweat avoid some of these challenges, but typically at the cost of lower target concentrations.

As nucleic acid-based sensors are implanted intravenously for continuous operation, the challenges of operation under continuous and variable blood flow are presented. Implantable sensors for circulating biomarker quantification must contend with the dynamic nature of blood and ISF, which can be influenced by hydration levels, blood pressure, and other systemic factors. Additionally, the integration of these sensors into the cardiovascular or lymphatic systems introduces risks associated with blood clotting and embolism. Therefore, the materials and the sensor's surface properties must be meticulously designed, like robust anchoring mechanisms that do not compromise the integrity or functionality of surrounding tissues to avoid triggering any adverse vascular responses. Moreover, to protect sensors from fouling, various methods are utilized to effectively prevent signal degradation in biological environments. These advancements enable promising prospects for long-term *in vivo* monitoring applications.

Overall, the development of implantable sensors requires addressing a complex array of challenges. These include ensuring accurate biomarker detection across a wide range of concentrations, maintaining sensor stability and calibration in a dynamic systemic environment, mitigating risks associated with sensor site implantation, and designing robust sensors without inducing an adverse foreign body reaction. Despite recent advancements in nucleic acid-based sensors, the quest for implantable probes that can provide continuous, real-time data on a wide array of circulating biomarkers with minimal operational challenges remains ongoing, underscoring the need for continued research and development in this critical area of personalized medicine.

5. Design considerations toward continuous *in situ* biomarker analysis

The primary objective driving the development of wearable and implantable sensors is to enable the continuous monitoring of biomarkers within body fluids. Successful and robust measurement of molecular biomarkers is challenging for several reasons, including the intricacies of multi-step bioaffinity assays, sensor regeneration, sensor stability, gradual biofouling of sensor surfaces, efficient transport of samples over the sensor, and calibration issues specific to on-body sensing. In this section, we will introduce the challenges associated with various systems and biofluids and discuss potential solutions and guidelines for the design of next-generation nucleic acid-based wearable and implantable biosensors (Fig. 7). We will provide insights into future research directions and the promising prospects within this dynamic research field toward seamless, continuous monitoring of biomarkers *in situ*.

5.1 Reagentless real-time sensing of ultralow-level biomarkers

Given that strategies designed for externally added reporter molecules may not be compatible with on-body based biomolecular sensing, we focus on reagentless sensing approaches aimed at sequential real-time monitoring of ultralow-level biomarkers. Ideal reagentless sensing seamlessly integrates the biorecognition event with signal transduction. Redox reporter-labelled aptamers undergo a binding-induced conformational change that alters the detected electron transfer rate. Redox reporter-labelled molecular pendulums exhibit changes in the detected electron transfer rate due to the drag force elicited by target binding. Selective molecular binding at the surface of the transistor gate redistributes surface charges amplified to a measurable source–drain current. These modular design schemes provide a basis for reagentless nucleic acid-based sensing.

Conventional nucleic acid-based sensor designs involving a redox reporter have relied on dual-frequency SWV measurements, which have been found to limit the time resolution of the sensor to approximately 6 to 22 s.[105] To accelerate electrochemical interrogation and further approach real-time sensing, fast Fourier transform electrochemical impedance spectroscopy (FFT-EIS) was used.[105] FFT-EIS simultaneously captures the impedance at multiple frequencies in under 2s to evaluate the electron transfer rate without the need for individual sensor calibration and drift correction. This finding emphasizes the need for ongoing exploration of analytical electrochemistry techniques in the biosensor space.

Transient circulating metabolites and trace biomarkers require ultrasensitive detection by implantable sensors. Additionally, non-invasive wearable sensing in peripheral biofluids often requires biomarker detection at low concentrations relative to blood.[11,12] Nucleic acid-based sensors have demonstrated excellent sensitivity in a variety of biofluids. Beyond the nucleic acid binding scheme, high efficiency electron transfer is key to the performance of the sensor. Nanostructured materials with high electroactive surface areas and charge carrier mobility improve the electrochemical sensor's SNR.[106] The presence of nanopores reduces the impact of charge screening, thus reducing Debye length limitations and enhancing signal gain.[107] Nanostructured gold electrodes for high performance nucleic acid-based sensors have been fabricated using a variety of methods, including inkjet printing of AuNPs, deposition of Au nanowire-polymer nanocomposites, electrodeposition of 3D Au nanostructures, electrochemical roughening, and selective etching of cosputtered Au alloys to form nanoporous Au.[36,44,107–109] Graphene among other semiconductor nanomaterials has also been exploited for its exceptional conductivity, flexibility, and biocompatibility, with many implementations in nucleic acid-based transistors.[27,30,48,90,110,111] MXene composites may further enhance electrode performance through the incorporation of transition metals and electrode modification with reactive functional groups.[44]

Signal amplification methods may also be used to detect ultralow-level biomarkers in real-time. Transistor-based sensors allow for ultrasensitive detection due to the signal amplification of the biorecognition event.[14] Enzymatic signal amplification has also recently been explored through the use of DNA aptamer-enzyme complexes.[112] Ultimately, sensitivity is limited to the affinity of the biorecognition element. This presents a key challenge in reagentless real-time biosensing

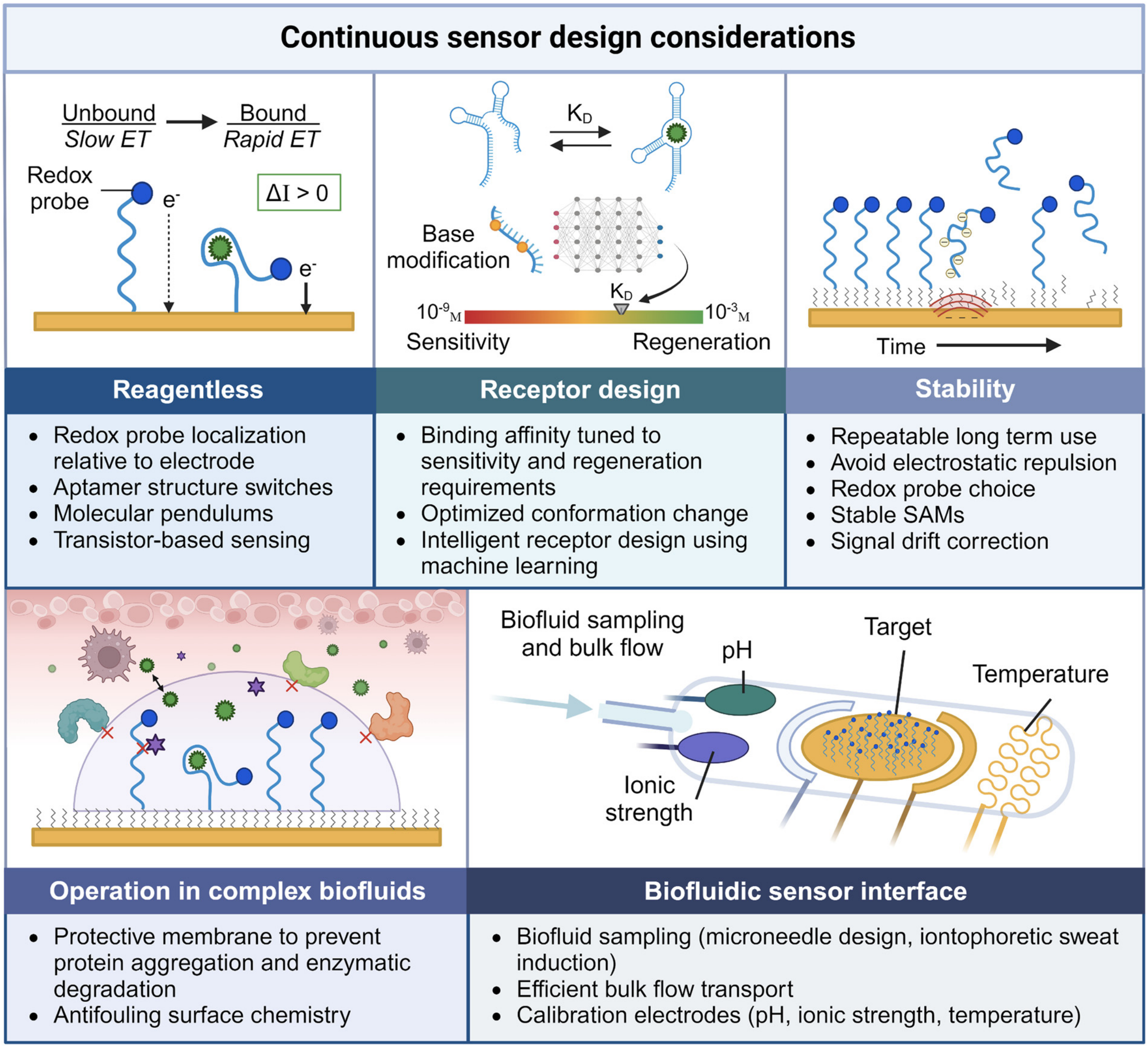

Fig. 7 Design considerations toward continuous, *in situ* and real-time molecular analysis.

of balancing high affinity ligand binding with bioreceptor auto-regeneration.

5.2 Bioreceptor design toward continuous monitoring

Nucleic acid receptor sequences are often selected using SELEX which favors the selection of high affinity receptors for optimal sensitivity and selectivity. However, for continuous sensing, reversible binding is necessary to capture fluctuating target concentrations. An apparent trade-off exists between receptor sensitivity and regeneration. Here, we deliberate on nucleic acid receptor sequence design according to dissociation kinetics.

Biochemical strategies to tune the nucleic acid dynamic range include sequence mutations, structural changes, and allosteric inhibition.[113,114] Taking a high affinity aptamer, the aptamer's bound state can be destabilized by making nucleotide substitutions, shortening the length of the terminal stem, and using a circular permutation of the sequence.[113] Intramolecular strand-displacement switch design was presented as a strategy to decouple aptamer thermodynamics and kinetics in an effort to tune the temporal resolution of the aptamer without reducing the effective binding affinity.[114] In this method, the native aptamer sequence is extended to a stem-loop structure with a poly-T linker and complementary displacement strand with fluorophore-quencher interactions informing target binding during stem-loop unfolding. Loop length, displacement strand length, and base-mismatch mutations were then used to tune the aptamer binding kinetics. Engineering aptamer dissociation kinetics *via* mutagenesis is difficult since the three-dimensional structure of aptamers is often unknown. Additionally, mutagenesis may result in changes in the specificity of the bioreceptor. To obviate changing the aptamer sequence, allosteric inhibition was explored to alter binding dynamics.[113] Oligonucleotide inhibitor strands bind to portions of the aptamer to stabilize the nonbinding configuration. This competitive binding between the oligonucleotide inhibitor and the target is easily optimized by tuning the concentration and complementary sequence length.

Machine learning has enabled *in silico* aptamer discovery. A machine learning-guided particle display methodology was developed to generate libraries of high affinity aptamers against a chosen target and furthermore truncate aptamer sequences while maintaining the affinity.[115] This methodology led to more and higher quality aptamer candidates. Another approach to aptamer discovery emphasized overall secondary structure by using a sequential multidimensional analysis algorithm for aptamer discovery from high-throughput SELEX pool sequencing data.[116] The algorithm evaluated motif and substructure patterns, aptamer family abundance, and secondary structure stability. Integrating algorithmic tuning of aptamer features into parallelized SELEX workflow can rapidly accelerate aptamer generation and selection to a matter of days.[117] Future multi-parameter machine learning models may allow for the simultaneous optimization of aptamer characteristics such as affinity, specificity, dissociation kinetics, structure, and stability, beyond current experimental capabilities. Advances in machine learning have also enhanced our working understanding of nucleic acid receptors *via* simulations for molecular docking and binding dynamics.[118–120] One challenge in engineering aptamer designs is in identifying the ligand-bound nucleic acid conformation, since the most thermodynamically stable conformation is not always in agreement with the highest affinity interaction.[121]

Molecular docking simulations have allowed for the identification of metastable structures leading to more accurate aptamer-target binding interaction predictions.[119] By understanding target binding interactions, we may rationally fine-tune nucleic acid receptor designs. The goal dynamic range for *in situ* sensing applications as well as the temporal resolution must be considered when engineering nucleic acid receptors for continuous biosensing. Further, oligonucleotide length, mismatched bases, and structure should also be examined to optimize the signal transduction of nucleic acid-based sensing schemes. While *in silico* modelling has enhanced aptamer discovery and conformation predictions, future work is needed to experimentally characterize aptamer structures and better understand nucleic acid-target interactions. Development of nucleic acid characterization techniques will help to validate predictive machine learning models.

5.3 Sensor stability

Sensor stability is a key characteristic for accurate continuous sensor measurements. Stability of wearable and implantable biosensors may be impacted by several factors including chemical stability of redox reporters, self-assembled monolayer (SAM) degradation, voltage-induced desorption of receptor elements, and biofouling.[122–124] Signal drift can be mitigated through thoughtful sensor design choices as well as algorithmic corrections. Developing stable nucleic acid-based sensors will be necessary for the translation of these sensors to implantable devices for long-term use.

A major design consideration for many electrochemical nucleic acid-based sensors is the choice of redox probe for enhanced electron transfer rate, signal quality, and stability. One of the most common redox reporters in the literature is MB, and for good reason, since MB demonstrates exceptional stability over time, under repeated voltametric interrogation, and under repeated nucleic acid sensor target regeneration.[124] MB also has a redox peak around −0.26 V, which is well below the redox potential for common conductive substrates, ensuring high SNR. However, the negative potential window of MB is a disadvantage for negatively charged nucleic acid-based sensors, due to voltage-induced repulsion and desorption. Stability experiments demonstrate that the more negative bias potential exposure the nucleic acid-based sensor experiences (*e.g.* low SWV frequency, high measurement frequency), the more the signal decays.[122] This suggests that redox reporters with a positive redox potential, such as a Fc derivative, merit further investigation for long term sensor stability.

Electrochemical nucleic acid-based sensors are often functionalized relying on solution-based processing to generate SAMs on the surface of electrode substrates, which ensures the uniformity of the DNA layer. The volume of the solution used for forming SAMs does not significantly impact the process. By utilizing uniform lengths of DNA, the thickness of the nucleic acid layer can be precisely controlled, allowing for a consistent detection layer thickness and the formation of fine-sized sensor patterns.[125,126] In contrast, drop casting techniques, widely used in electrochemical sensing, often suffer from issues such as the coffee ring effect, which can alter the distribution of enzymes drop-cast onto an electrode. Unlike the manual drop casting of enzyme films, the solution modification process for creating SAMs yields higher reproducibility, thereby reducing variability in sensor performance. This enhanced reproducibility is crucial for the reliable detection of chemical biomarkers, making SAMs a superior alternative for precise and consistent sensor fabrication.[127]

Passivation by alkylthiolate chains support the alignment of the nucleic acid monolayer and prevent non-specific redox reactions at the gold surface. These monolayers must withstand repetitive voltage perturbations as well as prolonged biofluid interactions. Ongoing work to improve upon the standard of mercaptohexanol has prioritized gold binding interactions and enhanced lateral van der Waals interactions between monolayer molecules.[128] Increasing the alkylthiolate length by two methylene groups demonstrated improved week-long stability at 37 °C.[128] Longer monolayer alkylthiolate chains may improve stability but at the cost of reduced electron transfer kinetics.[41] Crosslinking monolayers also increases the strength of the interactions between monolayer molecules to limit desorption susceptibility.[129] SAM stability may additionally be enhanced using multidentate anchoring groups to increase attachment points to the electrode surface.[129] The oxidation of thiol-based monolayers to disulfide dimers contributes to monolayer instability. Performing electrochemical scans in a negative potential range, while bad for the nucleic acid receptor, may actually suppress oxidation of alkylthiolate monolayers.[128]

N-Heterocyclic carbenes have recently been explored as an alternative to thiol-based monolayers with strong affinity to gold, robust monolayer stability, and efficient bioconjugation chemistry. However, N-heterocyclic carbene monolayers were found to desorb at voltages less than −0.1 V and above 0.25 V, thus limiting the potential scanning range for redox reporters.[130] Continued work is needed to explore new alternative passivation layers that promote long-term sensor stability in biofluids.

Beyond sensor design, signal drift can also be corrected for algorithmically, current methods including dual frequency measurements and ratiometric self-calibration. Aptamer-based sensors exhibit SWV frequency-dependent behavior. If the aptamer exhibits a concentration-independent signal response at a nonresponsive frequency, ratiometric corrections may be used to self-calibrate sensors, reducing interelectrode variability and correcting for drift.[43,131] For aptamers that exhibit a signal-on and a signal-off domain, the kinetic differential measurement (KDM) may be used to enhance the SNR and correct for signal drift assuming these two measurements exhibit matching background responses.[42,101] The KDM signal is obtained by taking the difference between the signal-on and signal-off SWV peak current measurements divided by their average. These algorithmic corrections require careful optimization of the SWV frequency, which varies between aptamers with structure and electron transfer kinetics.

Alternatively, internal controls may be incorporated into the sensor design for ratiometric self-calibration. Interrogating the response of a target-sensitive nucleic acid sequence and a non-binding oligonucleotide strand in either a dual-electrode or single-electrode system may inform the nucleic acid sensor's degradation over time. A ratiometric electrochemical aptasensor was designed to self-calibrate based on the combined signal from a Fc-labeled aptamer and a Fc-labeled DNA hybridized to the same template strand.[132] This technique demonstrated a more stable response under varying temperature and pH conditions than conventional EABs. Dual-aptamer measurements have been studied to improve stability by screening for and selecting aptamers with similar drifts.[133] Differential signal responses for self-calibration can also be achieved with adjustment of the redox reporter position or use of a secondary intercalated redox reporter.[133,134]

While these ''self-calibration'' techniques offer methods to correct for signal drift, they are a temporary fix to the stability problem of nucleic acid sensors. Current drift correction techniques lack generalizability across nucleic acid sensors with significant optimization required between sensors of different targets. Additionally, these techniques often require two SWV scans for a single measurement, thus reducing the time resolution and potentially accelerating sensor degradation. Future work is needed to further enhance the stability of nucleic acid-based sensors through rational design of redox reporters and stable electrode functionalization. We next discuss how stability is one of many issues exacerbated by operation in biofluids and implantation with *in vivo* long-term stability requiring that sensors overcome more biocompatibility challenges.

5.4 Operation in complex biofluids

Sensor operation in complex biofluids introduces challenges of high protein and cellular content, and variable media conditions (pH, ionic strength, temperature, interferents, *etc.*). Surface fouling is a major concern for any bioelectronic interface. When biomolecules in the sampled biofluid adsorb on the electrode surface, electron mobility is disrupted, impacting the signal transduction of the sensor. Fouling-induced desorption of sensor elements has been found to be a primary source of signal degradation of nucleic acid-based sensors in biological fluids.[123,128,135] Antifouling design considerations to enhance sensor stability in biofluids are described, including the use of porous membranes, hydrogel encapsulation, polymer coatings, SAMs, and nanoporous conductive substrates. Considering that body fluid compositions may vary intra- and inter-individually, we introduce methods to improve the sensor's performance across variable conditions, such as temperature, pH, and ionic strength.

During initial exposure of the sensor to biofluids, foulants occupy defects in the monolayer of the nucleic acid-based sensor often leading to a drop in signal. These foulants may then further accelerate desorption around the defect due to intermolecular interactions with the monolayer molecules, emphasizing the importance of a stable SAM.[128] Thiolated molecules present in biofluids, such as cysteine and glutathione, compete with gold-thiol bound nucleic acid bioreceptors resulting in monolayer displacement.[122] While non-specific adsorption of proteins on the sensor surface has demonstrated long-term stability by reducing monolayer desorption, unpredictable protein unfolding interactions may interfere with nucleic acid bioreceptors and constrain movement in conformation-switching sensors.[122,136]

In order to mitigate fouling, physical and chemical barriers may be used to protect the sensor. Applying a hydrogel membrane limits diffusion to small molecules as determined by the porosity of the hydrogel design. Polybetaine-, agarose-, and polyacrylamide-based hydrogels have been used to encapsulate aptamer sensors to prevent sensor signal degradation when deployed in biological tissue, serum, and cells.[104,128,137] Macroporous hydrogels embedded with aptamer-functionalized AuNPs have also been used to prevent foreign body rejection of implantable sensors.[138] Porous conductive substrates, such as nanoporous gold and gold nanocomposites, dually benefit from efficient electron transfer as well as antifouling properties.[107,109,139] Semi-permeable polymer coatings like Nafion also provide antifouling properties and may prevent small molecule interferents from contacting the sensor.[140–142] Zwitterionic monolayers have demonstrated reduced protein adsorption. For example, phosphatidylcholine-terminated SAMs promote an antifouling surface chemistry by mimicking the cell membrane.[143,144] With these antifouling methods, week-long *in vivo* nucleic acid-based sensing is a feasible goal that may soon be realized.[128]

As the sensor stability improves past several weeks, implant device form factor must also be considered to prevent foreign body reaction and encapsulation. Limiting protein aggregation through antifouling techniques evades the attraction of immune cells to the device. Minimizing implant size and matching the Young's modulus of the surrounding soft tissue with viscoelastic, soft electronics may help address this problem. Chemical non-toxicity of the sensor's components is important to prevent localized necrosis and subsequent immune responses. Mechanical strength of the sensor and resistance to *in vivo* shearing and degradation is key to prevent long-term toxicity through particulate accumulation in distal organs such as the liver and kidneys. Careful consideration must be taken for the types of nanomaterials used in the sensor design.[145]

Although blood is one of the most complex biofluids from a fouling standpoint due to its biomolecular diversity, circulating blood conditions are homeostatic resulting in consistent narrow ranges of variability over time and between individuals. ISF and CSF are filtered blood derivatives for wearables, subcutaneous implants, and neural implants that also maintain essential homeostatic conditions. However, when considering alternative biofluids, such as sweat, saliva, intestinal fluid, and urine, media conditions must be monitored as they may vary significantly between samples. In the case of sweat, pH may vary widely from 5 to 9 due to sweat induction methods, sweat rate, and individual physiochemical response.[12] Sweat ion concentration also varies with sweat rate. Sweat as a highly filtered, yet variable biofluid presents opposing design challenges to blood.

To account for variable media conditions, the nucleic acid-based sensor must be robust and operable under wide-ranging biochemistries. pH may significantly impact electron transfer kinetics, so pH-insensitive redox reporters have been studied to handle variable media conditions.[146] Electrochemical nucleic acid-based sensors have improved signal transduction at decreasing ion concentrations, as is the case with sweat, due to reduced charge screening effects.[107] As mentioned previously, dual-nucleic acid measurements can help to reduce the sensor variation to environmental factors, such as pH, ion concentration, and temperature.[132,133] However, in most cases, variable sensor responses will need to be calibrated using a multiplexed sensing platform that monitor these media conditions simultaneously.

5.5 Biofluidic sensor interface

The performance of wearable and implantable sensors is heavily influenced by how they interface with dynamic and diverse compositions of biofluid matrices. The sensing platform efficiently samples and conducts the biofluid to the surface of the nucleic acid-based sensor *via* layered microfluidics. The *in situ* sensing platform may contain several sensors for calibration and multibiomarker analysis also interfacing with the sampled biofluid. Minimally invasive, flexible form factors are key elements for consideration when designing the sensing platform for wearable and implantable applications.

As mentioned previously, nucleic acid-based sensors may require multiplexed platform calibration to account for variable conditions such as temperature, pH, and ionic strength, especially when working with blood-alternative biofluids. Electrode impedance can be used to measure ionic strength. A polyaniline-based potentiometric pH sensor was used for sweat calibration in a wearable ring platform.[44] When fabricating aptamer-FET sensors, it may be convenient for associated platform sensors to also be transistor-based, so a FET pH sensor was developed for a flexible FET biosensor array for real-time cortisol sweat sensing.[46]

While implanted sensors are under constant exposure to biofluids, wearable sensors must non-invasively capture the biofluid. Janus membranes have been studied for ultralow volume biofluid sampling. The asymmetric wettability of porous Janus membranes enables unidirectional liquid transport and self-pumping for high efficiency biofluid collection.[147] Janus membranes are an attractive strategy for passive natural sweat collection without external stimuli (exercise, temperature) at low sweat rates.[148] They also aid in filtering out large insoluble impurities often present on the skin's surface. Sweat may also be stimulated using cholinergic agents delivered *via* iontophoresis.[44] Microfluidic inlets conformed to the skin may then effectively capture sweat from the local stimulated area. Microfluidic designs may provide important control over the biofluid sensor interface. The microfluidics for a singular sensing region in a label-free continuous sensing platform may be simple when it comes to fluid transport, but it is important that the refreshing dynamics of the sensing region are evaluated and microfluidic inlet and outlet design optimized.[149] In other platforms, CBVs may be necessary for microfluidic gating to control the reservoir filling order.[150] This can be used to provide chronologic sampling of a biofluid without mixing of old and new samples. This technique can be used to capture multiple measurements with a sensor array on a single platform thus leveraging a single-use sensor for continuous monitoring. CBVs may also be used to control fluid flow for incubation in the sensing region. A wearable platform for female hormone analysis used microfluidic CBVs to precisely control sweat sampling during a strand displacement assay.[44]

Soft, flexible electronics are key to interfacing sensors with tissues. Wearable sensors must conform to the complex wrinkles of the skin, curvatures of the body, and withstand repeated strain and bending deformation associated with body movement. Adopting mechanical properties that mimic the elasticity of skin allow the sensor to remain in position for localized sample collection and maintain performance for continuous sensing. Flexible thin-film polymer substrates, stretchable conductive polymer composites, liquid metal-based conductors, flexible graphene electrodes, strain-releasing lead patterns, and bioadhesives are some strategies used towards a tissue conforming sensing interface.[148,151–156] Implantable sensors must interface well with the surrounding tissue and demonstrate strong biocompatibility to prevent encapsulation that would limit the long-term performance of the sensor.[82] Beyond flexibility, soft electronics with macroporous scaffolds are key for vascularization and tissue integration. For example, embedding AuNPs into a hydrogel improved the tissue response to two months of implantation with minimal fibrous encapsulation.[138] Minimizing the size of the sensor may also reduce the risk of a foreign body reaction. Thin, flexible conductive fibers are a promising direction for implantable sensing due to the matching mechanical properties for *in vivo* use.[82] For example, a graphene-elastomer composite neurotransmitter sensor called NeuroString was fabricated as long strings for brain implantation *versus* as a thin film for use in the larger gut cavity.[153] The current response decreases with size for sensors that rely on 1 : 1 redox probe labelled nucleic acid signaling strands. The amplification provided by nucleic acid-based transistors thus bolsters the use of transistor design schemes for miniaturized implantable sensors.[89,90]

Using multiplexed sensor arrays, spatial information may also be collected at the tissue interface. EAB sensors were placed at multiple sites along a probe implanted into tumor tissue for radial measurements of chemotherapeutic drug delivery and uptake within the tumor.[103] Wearable microneedle array patches allow for high density spatial sensing across the skin surface but may not reveal varied spatial results beyond local epidermal health (melanoma, wound healing). Tissue-conforming meshes allow for 2D measurement, which may elucidate important signaling and activity information when interfaced with the brain and heart.[154,156] Nucleic acid-based microtransistor arrays will be the optimal strategy to achieve high density spatial resolution since transistors maintain an amplified signal upon miniaturization. Spatiotemporal data may also be collected using smart pill designs in which the ingestible capsule collects data as it naturally moves through the digestive system.[157,158]

From biofluid sampling to sensor calibration, rational design decisions must be made to support the translation of nucleic acid-based sensors to wearable and implantable devices. While nucleic acid receptors have the potential to be selective for diverse small molecule targets, these receptors must be engineered with regeneration in mind. Continuous monitoring requires kinetically favorable receptors and sensing mechanisms capable of detecting fluctuating analyte concentrations without user intervention. Yet, the primary challenge to overcome in continuous nucleic acid-based sensor design will be long-term stability. Compatible design choices of redox reporter, passivation layer, antifouling membrane, and electrochemical interrogation methods will extend the stability and robustness of nucleic acid-based sensors for effective continuous use in complex biofluids. Given the challenges in realizing continuous long-term sensing, disposable sensor patches with sensor arrays may be a more attractive and realistic translation of nucleic acid-based sensors for wearable platforms. However, for sensor implants to be worthwhile, these design challenges will need to be overcome for implantation without the need for frequent surgical replacement.

6. Conclusion

The development of continuous implantable and wearable electrochemical sensors represents a major advancement in the field of precision medicine and personalized healthcare. For example, implantable and wearable sensors may provide key information for effective drug delivery and dosing based on individual pharmacokinetics. Implantable and wearable devices may synergistically be used to implement closed-loop therapeutic systems with *in vivo* data access and noninvasive modulation. In addition, continuous monitoring using implantable and wearable sensors may provide advanced diagnostic data with respect to personalized baselines and trends that would allow for early intervention. This review has showcased the potential of nucleic acid-based sensors to provide continuous, real-time monitoring of a broad spectrum of biomarkers directly in various biofluids. By leveraging the high specificity and sensitivity of the affinity of nucleic acids towards target biomarkers, these sensors offer a promising solution to the limitations of traditional health monitoring methods, which are often intermittent, invasive, and unable to capture the dynamic nature of physiological changes.

We have delved into various nucleic acid sensing strategies, with a particular focus on nucleic acid-based recognition elements and their role in enhancing the performance of biosensors. The integration of these sensors into both implantable and wearable platforms has been discussed, demonstrating their versatility and potential for widespread application in continuous at-home health monitoring. We critically evaluated challenges such as ensuring sensor longevity, navigating the intricacies of biofluids, and achieving reagentless detection, and proposed innovative interdisciplinary solutions to address these issues. Continuous efforts to improve sensor design, along with advancements in materials science, bioengineering, and computational modeling, are imperative for overcoming current limitations and enhancing the functionality and reliability of these devices. The integration of these sensors with digital health platforms could further enable real-time data analysis, fostering a proactive approach to health management and disease prevention. As we navigate the complexities of their development and implementation, the promise of transforming personalized medicine into a tangible reality becomes increasingly attainable, heralding a new era of health monitoring and intervention.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

Conflicts of interest

The authors declare no competing interests.

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