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RESEARCH

Dual-cytokine profiling of inflammatory states using a rapid electroanalytical device as a point-of-care sensor platform

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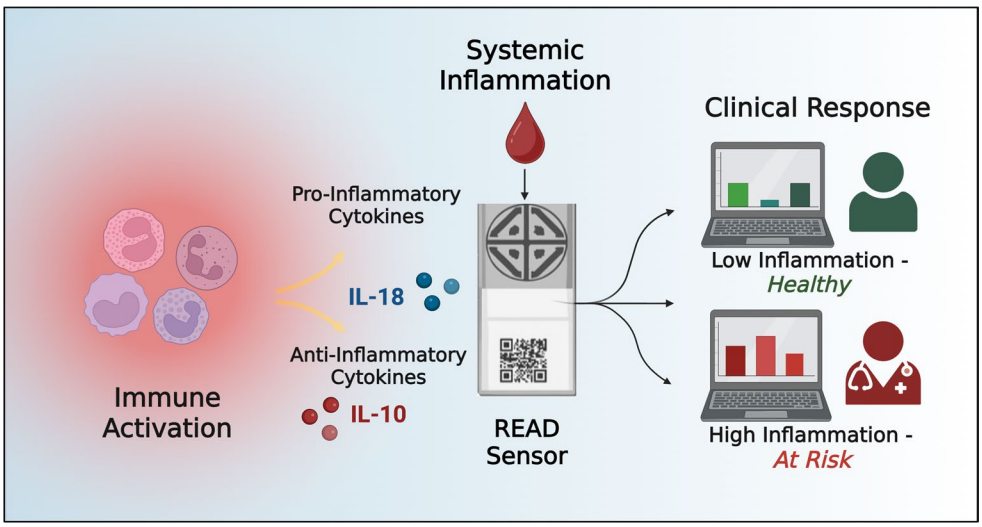
Abstract

The dynamic mechanism of inflammation plays a pivotal role in the pathogenesis of many acute and chronic disease states, with interleukin-18 (IL-18) and interleukin-10 (IL-10) as important markers representing pro-inflammatory and anti-inflammatory responses, respectively. An imbalance in the levels of these cytokines can worsen disease severity and patient outcome, highlighting the need for tools that simultaneously detect and quantify levels for diagnostic purposes. This study uses the READ (Rapid Electrochemical Analysis Device) platform, an innovative biosensor technology, utilizing the electrochemical impedance spectroscopy (EIS) technique, which is equipped to quantify IL-18 and IL-10 levels directly in human blood samples. The READ platform offers sensitive, label-free detection of inflammatory markers with a rapid processing time (< 10 min) and requires minimal sample volume (≤ 160 µl). The device exhibited a robust analytical performance, with a strong dose-response linearity (R^2 = 0.98–0.99), consistent recovery (80–120%), and minimal cross-reactivity across cytokines (IL-18 and IL-10) and across matrices (K_2-EDTA whole blood and plasma). The portable, user-friendly approach to the READ platform bridges the gap between tedious laboratory processing and clinical-level field deployment, thus paving the way for personalized inflammatory disease management. This work highlights a transformative approach to cytokine monitoring, allowing for early intervention and improved therapeutic outcomes for patients fighting inflammatory states.

Graphical abstract



Keyword Cytokines · Multiplexing · Biosensing · Electrochemical · Impedance · Immunosensor

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Introduction

The inflammatory process

Inflammation is a rather complex physiological process triggered by harmful stimuli, including but not limited to foreign pathogens, invading toxins, or cell damage remnants. This process is critical in regulating tissue integrity and host defense mechanisms. The mechanism of inflammation is rather complex, with various pathways, fighters, and regulatory processes associated with repair mechanisms [1]. While acute inflammation is essential in eliminating infections and rapid initiation of repair mechanisms, chronic inflammation is a hallmark of various disease states, such as autoimmune conditions, metabolic syndrome, cardiovascular and respiratory diseases, and certain cancers [2]. These resulting chronic inflammatory conditions are the most significant cause of death worldwide [3]. According to the World Health Organization (WHO), chronic diseases are the greatest threat to human health [4]. The inflammatory process is tightly regulated by means of a balance between both pro-inflammatory and anti-inflammatory molecules, known as cytokines, chemokines, and other regulatory immune cells. These collectively orchestrate the dynamic immune signaling and resolution pathways. When the equilibrium between both regulatory processes is disrupted, persistent inflammation may lead to tissue damage and disease progression. Thus, it is important to monitor inflammatory biomarkers to better understand the onset of diseases through early diagnostics and allow for the rapid initiation of therapeutic intervention [5].

Role of interleukin-18 and interleukin-10 in inflammation

Two key drivers in the diverse network of cytokines are interleukin-18 (IL-18) and interleukin-10 (IL-10). The markers represent two critical yet opposing regulators of physiological immune system function. IL-18 is a pro-inflammatory cytokine that enhances interferon-γ (IFN-γ) production by T cells and natural killer (NK) cells. IL-18 subsequently amplifies immune activation and contributes to tissue damage in chronic inflammatory states [6, 7]. Elevated levels of circulating IL-18 are strongly associated with inflammatory states, including Crohn's disease in inflammatory bowel disease, systemic lupus erythematosus, and rheumatoid arthritis, where it can heighten disease severity by natural killer cell recruitment and macrophage activation [7, 8] Conversely, IL-18 is an anti-inflammatory cytokine that suppresses the expression of various pro-inflammatory immune fighters. This consequently limits antigen presentation and promotes tolerance of the immune system [9]. The dysregulation in IL-10 activity has been seen in uncontrolled inflammation and autoimmune diseases, such as psoriasis and inflammatory bowel disease [10]. This complex interplay between IL-18 and IL-10 exemplifies the delicate balance in inflammatory process signaling, as an imbalance in either marker may tip the immune system towards chronic inflammatory pathology (Fig. 1). Both biomarkers have attractive potential as prognostic and diagnostic biomarkers for monitoring inflammatory disease progression.

Point-of-care biosensing for cytokine detection

Due to the high sensitivity and specificity, conventional detection methods, including enzyme-linked immunosorbent assays (ELISA) and multiplex immunoassays, are the current gold-standard cytokine detection methods. These assays are limited in terms of long sample processing time, labor-intensive processes requiring trained personnel, and the need for centralized laboratory infrastructure for processing. This often leads to a delay in results, limiting the diagnostic potential of such assays [11, 12]. This delay increased the need for faster, more reliable, and better accessible cytokine monitoring tools, thus triggering interest in point-of-care biosensing tools. Electrochemical biosensors, specifically those utilizing electrochemical impedance spectroscopy (EIS), provide a promising tool for rapid cytokine panel testing. EIS is a label-free approach for cytokine detection in biological fluids, that enables sensitive monitoring of biomolecular interactions at the sensor electrode-to-electrolyte interface without the need for extensive sample preparation [13]. These sensors provide a cost-effective, portable deployment option in decentralized and patient-care settings. Challenges in these sensor devices lie in developing robust sensitivity and accuracy of detection across complex biofluids. Often, inflammatory conditions are not defined by single marker elevation or depression; thus, multiplex paneling is essential, yet potentially problematic in these POC devices. By addressing and improving these limitations, the world of electrochemical point-of-care sensors can broaden the potential of EIS-based biosensors to monitor cytokines like IL-18 and IL-10 in real-world settings.

This research addresses this gap by developing a dual cytokine biosensing platform capable of detecting IL-18 and IL-10 simultaneously. Using the EIS technique, this approach aims to provide rapid, label-free, and multiplexed detection directly from complex biological matrices, enabling timely monitoring of inflammatory status and supporting clinical decision-making in acute and chronic disease management.

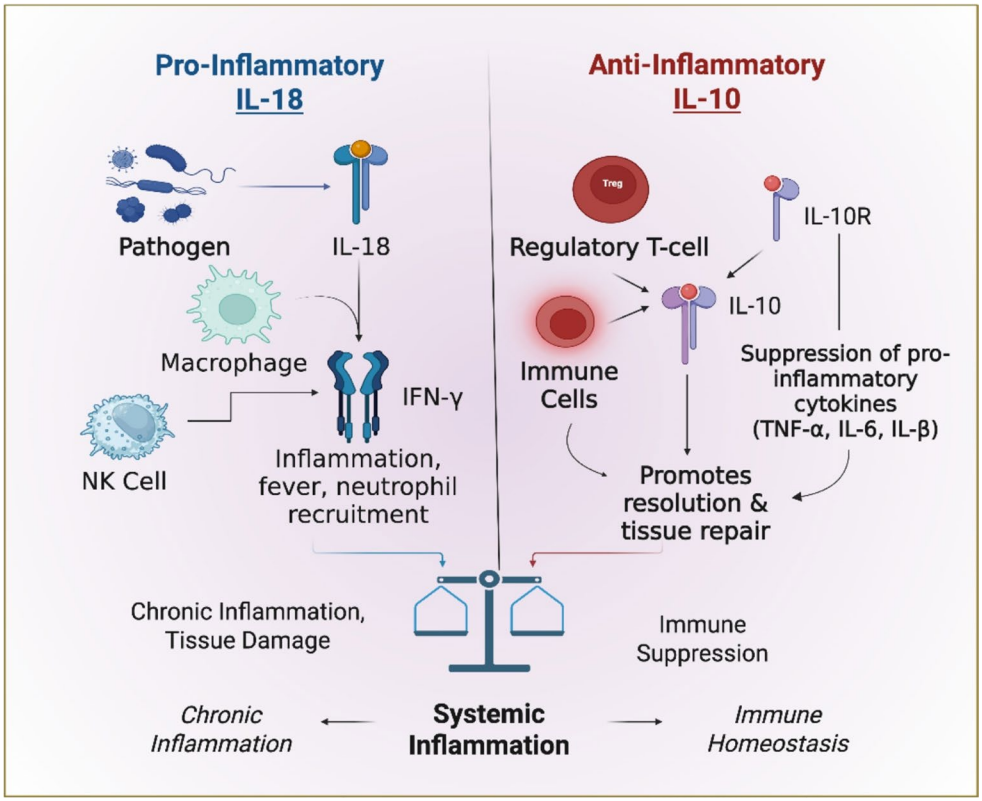

Fig. 1 Pathophysiological roles of pro- (IL-18) and anti-inflammatory (IL-10) cytokines in systemic inflammation

Materials and methods

Materials and reagents

Reagents, including monoclonal antibodies and respective recombinant antigens for Interleukin-18 (IL-18) and Interleukin-10 (IL-10), were purchased from R&D Systems (Minneapolis, MN, USA). The crosslinker, Dithiobis(succinimidyl propionate) (DTSSP), phosphate-buffered saline (PBS), and SuperBlock™ blocking buffer were purchased from Thermo Fisher Scientific (Waltham, MA, USA). K_2-EDTA whole blood samples were procured from Carter BloodCare (Bedford, TX, USA), and plasma was extracted from the whole blood samples via centrifugation. The blood samples were purchased for testing in sensor calibration and validation development. All procured reagents were of analytical grade and were used without further manipulation. According to manufacturer recommendations, stock reagent solutions were reconstituted and stored at -20 °C. Reconstituted aliquots, used for serial dilutions in K_2-EDTA whole blood and plasma samples, were thawed to room temperature for sample preparation and were limited to a maximum of three freeze-thaw cycles to prevent denaturation and maintain stability.

READ sensor design and measurement strategy

This study investigates the potential of the Rapid Electrochemical Analysis Device (READ) sensor platform for the concurrent detection of IL-18 and IL-10 in human whole blood and plasma samples. The READ platform is a compact biosensor system that includes a handheld electronic reader and a disposable sensor chip. The sensor chip is housed in a cartridge-style interface, which ensures reliable electrical contact with the reader through precise electrode alignment. This cartridge is designed to hold small volumes of biological samples on its surface. Electrochemical impedance spectroscopy (EIS) measurements were carried out using the portable electrochemical reader integrated into the system after the chip was placed in the cartridge, which helps to minimize contact resistance during measurements (Fig. 2).

The READ chip features 16 individual sensing electrodes on its surface, all contained within a cartridge for ease of use in clinical settings. Each sensor is made up of a gold working electrode, a gold reference electrode, and a carbon counter electrode. The working electrode surface was modified with a semiconducting zinc oxide (ZnO) layer to increase sensitivity for detection and facilitate biomolecular immobilization. This layer works by increasing the effective surface area for

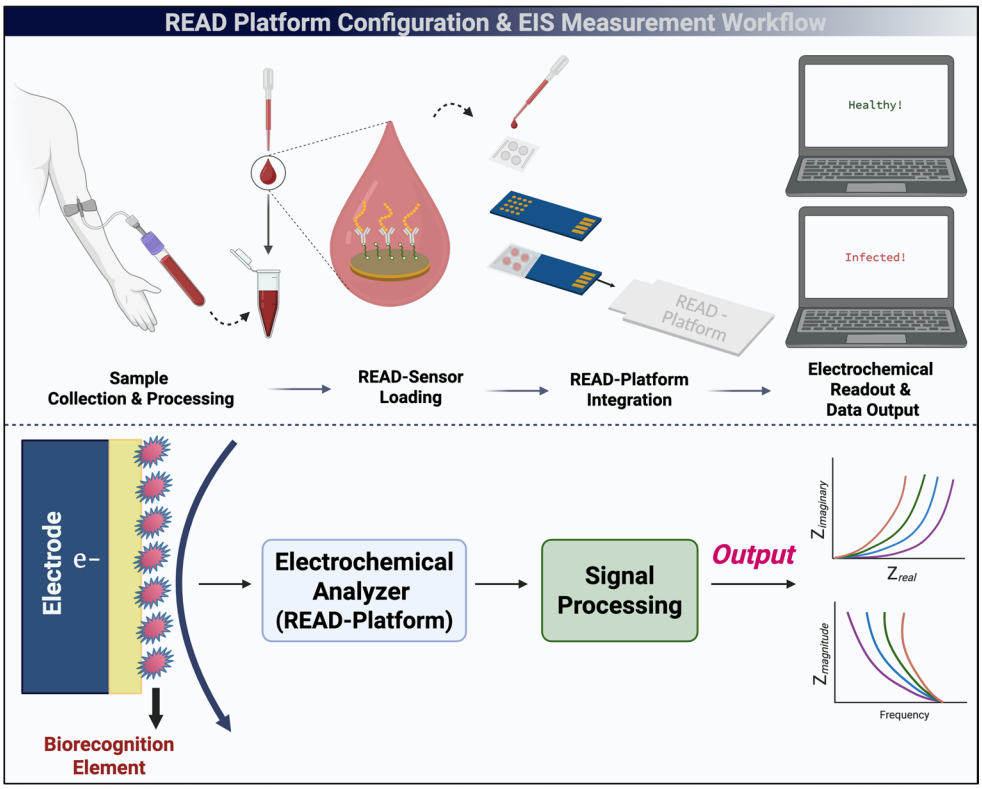

Fig. 2 Schematic illustration of the READ-platform configuration and electrochemical measurement workflow

binding and improving the potential of the immobilized capture antibodies to orient on the sensor surface properly. The working electrode performance was improvised using a semiconducting ZnO layer to enhance its sensitivity to biomolecular recognition events. The selection of semiconducting ZnO layer over a conducting modification layer was informed by its capability to offer adjustable interfacial resistance and capacitance, which are highly responsive to variations in surface charge. Upon antibody-antigen binding, the surface charge density undergoes a shift, influencing the depletion layer within the ZnO film and resulting in notable changes in charge-transfer resistance and interfacial capacitance, as seen through EIS. In contrast, conducting layers typically mitigate these disturbances due to their efficient charge transport properties. Furthermore, the ZnO layer increases the effective surface area and provides an optimal bio-interface for antibody immobilization, thereby enhancing sensor performance.

Previously, the READ sensor has been utilized in this laboratory to detect biomolecules, but employed a two-electrode system, a gold working electrode, and a gold reference electrode [14, 15]. The sensor design has since been modified to include a reference electrode to provide a more stable and reproducible reference potential. A carbon reference electrode was utilized to minimize polarization effects, resist reactions on the surface, and improve stability of the electrochemical system. Often, gold pseudo-reference electrodes lead to fouling and drift in complex biofluids, thus making the previously established system more difficult in blood-based biofluids [16, 17]. The device is integrated with wireless connectivity for automated data acquisition, transmission, and analysis via a computer system. EIS measurements were conducted by applying a small-amplitude sinusoidal perturbation over a frequency range of 80 Hz to 1000 Hz and resulting impedance measurements were analyzed. Impedance spectra were collected following surface functionalization, sample incubation, and antigen binding. All measurements were performed at room temperature under ambient conditions.

The device is small and portable, fitting in the palm of your hand, and the data is easily and rapidly collected on any electronic device. This refined, three-electrode system ensures more accurate impedance measurements, thus improving the sensitivity and reliability for biomarker detection in human biological samples. The design of the READ sensor uses electrochemical measurements to offer a more sensitive and translatable approach towards diagnostic devices.

Modification of electrode sensing platform

The semiconducting layer was deposited directly onto the gold working electrode surface prior to biomolecular functionalization, forming a stable semiconducting interface for subsequent crosslinker and antibody immobilization. The ZnO-modified gold working electrodes were modified with a sequential series of functionalization agents to quantitatively measure the presence and amount of each cytokine in a

patient's blood. The electrode surface was immobilized using a liquid-handling robot to automate the surface modification, allowing for precise reagent handling while reducing manual pipetting errors. This was completed for each sensor functionalization to ensure reproducible functionalization across multiple sensors. The immobilization process started by adding a cross-linker molecule (dithiobis(succinimidyl propionate) (DTSSP) for stable covalent conjugation of capture antibodies onto the ZnO-modified gold sensor surface. The cross-linker molecule was incubated with the respective antibody of interest at room temperature to promote amine-reactive conjugation. After the incubation, the DTSSP-antibody solution was equally dispensed onto the working electrodes and again incubated for 60 min to facilitate binding. On the 16-electrode READ sensor chip, half of the electrodes were modified with IL-18 antibody and the other half with IL-10 antibody for two-plex detection. Once immobilized, the electrodes were gently rinsed with deionized water to remove unbound antibodies and crosslinker, and a blocking agent was added, using SuperBlock™ blocking buffer for < 15 min. This buffer minimized non-specific binding and background noise, thus clarifying the desired impedance signal. The electrodes were again washed with deionized water to remove excess blocking agent, air-dried, and kept for lyophilization, or freeze-drying, at a temperature below −15 °C, for 25 min to maintain antibody stability during storage.

Sample preparation

The recombinant IL-18 and IL-10 antigens were spiked into K_2-EDTA whole blood and plasma samples over a clinically relevant concentration range for antigen sample preparation. Each functionalized and lyophilized electrode was exposed to 10µL of the prepared antigen-spiked blood sample, each, and incubated for 7 min at room temperature to initiate antibody-antigen binding interactions. Whole blood was applied to 8 of the 16 electrodes and plasma to the remaining eight electrodes, totaling four electrodes per biomarker per matrix. This structure for functionalization and testing was done to demonstrate the sensor chip's feasibility of capturing multiple cytokines and biofluids on a single chip using the sensor-cartridge configuration.

Results and discussion

Characterization of the 2-plex sensor

Zeta potential

Zeta potential is a technique used to define the potential difference between the adsorbed fluid layer (electrolyte) on a solid surface (electrode) and the bulk phase of the fluid, thus serving as an indicator of the mutual repulsion between the nanoparticles on the sensor surface and the stability of colloidal suspensions [18]. Zeta potential measurements were taken to evaluate the electrical potential of the slipping plane by measuring the electrostatic properties and colloidal stability of the sensor interface at varying concentrations. Measurements of the capture antibodies (IL-18 Ig-G and IL-10 Ig-G) were taken to assess the impact of antibody charge distribution. Then, the antibodies were incubated with the respective target antigens (IL-18 Ig-G + Ag and IL-10 Ig-G + Ag) at low, medium, and high concentrations to examine how antigen binding affects surface potential (Fig. 3A). The zeta potential for the antibody complexes (IL-18 Ig-G and IL-10 Ig-G) was measured at − 14.53 mV for IL-18 and − 10.53 mV for IL-10, reflecting the immobilization of antibodies and their influence on interfacial charge distribution. Following the incubation of the antibody solution with antigen solution at low, medium, and high concentrations (IL-18 Ig-G + Ag and IL-10 Ig-G + Ag), there were progressive changes in zeta potential ([low = -13.07, -17.36] mV, [medium = -19.66, -20.26] mV, [high = -23.73, -23.16] mV) in IL-18 and IL-10, respectively. These concentration-dependent shifts indicate antigen binding to the antibody, as the potential turns more negative, corresponding to enhanced electrostatic repulsion and increased suspension stability. Overall, the observed zeta potential changes confirm biomolecular interactions' binding and validate the functionalization process for IL-18 and IL-10 detection.

Fourier-transform infrared (FTIR) spectroscopy

To further validate the efficacy of the sensor functionalization, Fourier-transform infrared (FTIR) spectroscopy was used. FTIR spectroscopy functions by probing the rotational and vibrational transitions of molecular binding by measuring the absorption of infrared rays across a wide spectral range, yielding a molecular fingerprint of surface functional groups [19]. In this study, FTIR was carried out using a glass slide substrate coated with a thin zinc oxide (ZnO) film, modified with a crosslinker (DTSSP) and subsequently conjugated with capture antibodies (IL-10 Ab or IL-18 Ab), followed by incubation with the respective antigens (IL-10 Ag or IL-18 Ag). The FTIR spectra for each modification step are illustrated in Fig. 3B.

Fourier Transform Infrared (FTIR) spectroscopy was utilized to confirm the sequential surface modification of the ZnO electrode and the effective bioconjugation of functional elements on the sensor's surface. The spectra, illustrated in Figure 3B, display distinct absorption bands corresponding to each phase of the surface functionalization process: ZnO modified with the DTSSP linker,

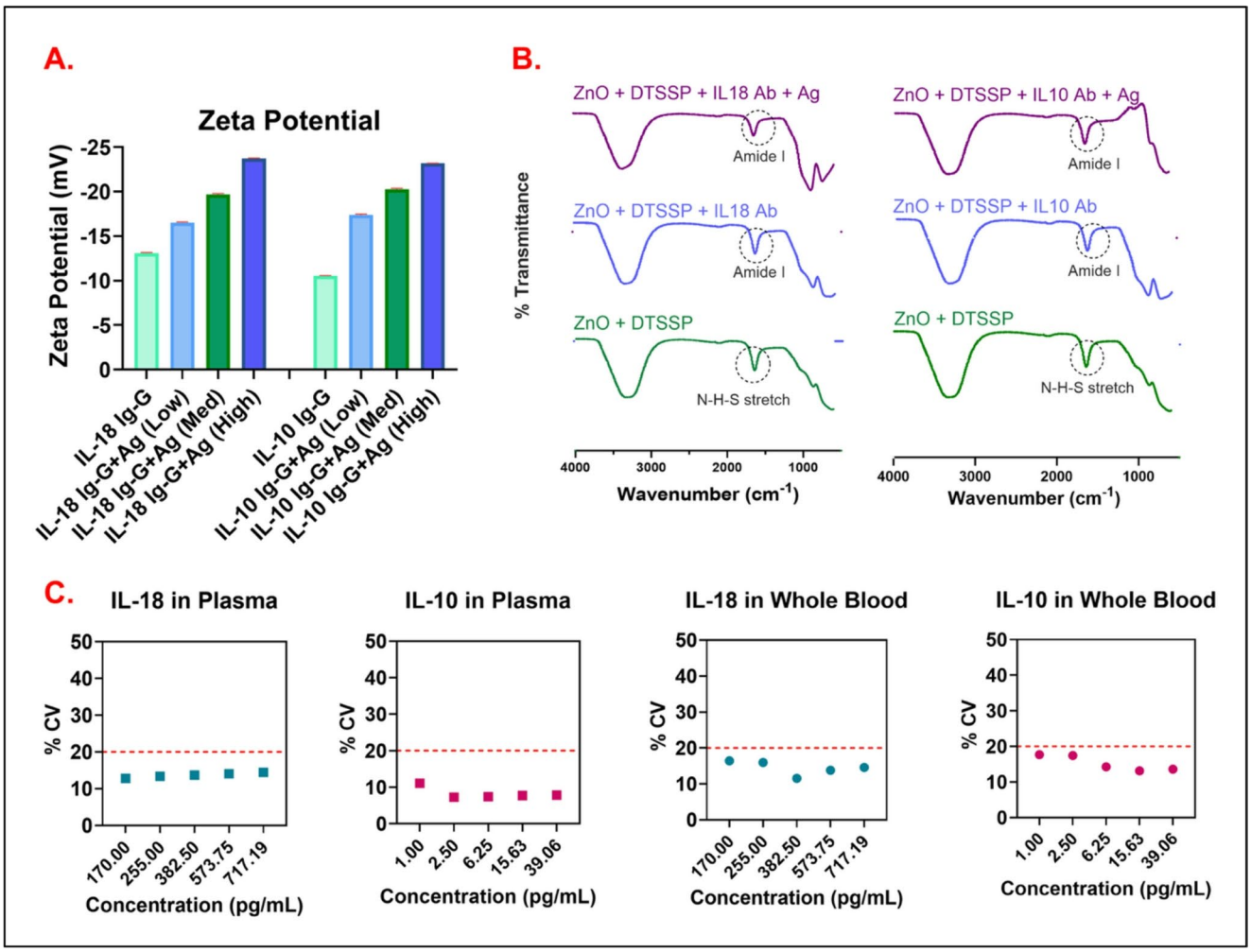

Fig. 3 Biophysical characterization of READ Sensor. **A** (Top, left) Zeta potential (mV) of IL-18 and IL-10 antibody complexes measured with antibody alone and after low, medium, and high antigen loading. **B** (Top, right) FTIR Spectroscopy highlighting characteristic bands and spectral shifts associated with antigen binding to confirm structural and conformational changes upon complex formation. **C** (Bottom right) Intra-assay variability (%CV) of IL-18 and IL-10 impedance responses measured by electrochemical impedance spectroscopy (EIS) under baseline (non-spiked) conditions

antibody (Ab) immobilization, and antigen (Ag) binding. The spectrum of ZnO with 3,3'-dithiobis(sulfosuccinimidyl propionate) (DTSSP) exhibited peaks around 1,650 cm^{-1} and 1,530 cm^{-1}, corresponding to the C=O stretching and N–H bending vibrations of the amide groups in DTSSP. These bands indicate the presence of reactive succinimidyl ester groups capable of covalently attaching proteins. Following antibody immobilization (ZnO+DTSSP+Ab), absorption bands appear around 1,640–1,660 cm^{-1} and 1,540–1,550 cm^{-1}, corresponding to the amide vibrations of peptide bonds, respectively. These distinctive peaks confirm the presence of protein biomolecules attached to the surface, demonstrating successful antibody coupling through the amide bond formed between the DTSSP ester and the antibody's amine groups. Upon antigen (Ag) binding (ZnO+DTSSP+Ab+Ag), the amide bands become more distinct and exhibit slight changes in both intensity and wavenumber. These alterations indicate conformational changes associated with the formation of the antibody–antigen complex. This spectral change verifies the specific biorecognition event and the successful capture of the target analyte on the biosensor surface. The FTIR spectra demonstrates that the crosslinker allows covalent antibody immobilization and that the READ platform successfully captures IL-18 and IL-10 antigens via affinity binding. These sensor characterization studies confirm the reliability of surface chemistry for cytokine detection.

Intra-assay variability

To evaluate repeatability and precision of the sensor, intra-assay variability was assessed using baseline conditions

(non-spiked, neat whole blood and plasma) for both IL-18 and IL-10. Intra-assay variability was evaluated using electrochemical impedance spectroscopy (EIS) by comparing impedance responses across replicate electrodes under baseline (non-spiked) conditions. Eight replicate electrodes were tested for each cytokine ($n=8$ for IL-18 and $n=8$ for IL-10, each in whole blood and plasma) on a 16-electrode READ sensor chip. The coefficients of variation (% CV) across the replicates were consistently below 20%, across both markers, thus confirming low signal response fluctuation at baseline (Fig. 3C). Intra-assay variability values for IL-18 and IL-10 are provided in Supplementary Table 1. Due to the complexity of whole blood, the variation is slightly higher compared to the response in plasma. This is likely due to the intrinsic complexity of whole blood, as cellular components, proteins, and other macromolecules contribute to non-specific interactions and steric hindrance, thereby increasing the variability in sensor response to whole blood, relative to plasma. Despite the higher %CV in whole blood, both markers meet the ≤20% coefficient of variation (CV) acceptance limit in both matrices. This confirms the repeatability and stability of the READ sensor during sensor characterization.

Electrochemical signal response on the READ two-plex sensor platform

Electrochemical impedance spectroscopy was utilized for the transduction method due to its high sensitivity in detecting interfacial changes occurring during binding [20–22]. Unlike other approaches, EIS monitors changes in charge transfer resistance and double-layer thickness, measured as double-layer capacitance, and provides a real-time assessment of antibody-antigen interactions without needing tags [22, 23]. EIS measurements in complex biological matrices, such as whole blood and plasma, are sensitive to matrix-dependent factors, including ionic strength and nonspecific adsorption, which can influence baseline impedance responses. In this study, these effects were mitigated by using matched biological matrices, surface blocking, and analysis based on relative impedance changes following antigen binding.

This highlights how advantageous EIS is for point-of-care testing in biofluids, as nonspecific background signals complicate various alternative methods. This technique is beneficial in patient-centered settings where clinicians or patients can easily use the sensor platform, as it eliminates the need for skilled personnel for handling, excess processing, and cumbersome sample collection from the patient. EIS is widely reported in biosensing literature as it is a tool compatible with miniaturized sensor architecture that provides rapid and reproducible results for clinical decision making [22].

The electrochemical impedance spectroscopy (EIS) technique was used to evaluate the electrochemical performance of the READ two-plex sensor for simultaneous detection of IL-18 and IL-10 in spiked K_2-EDTA whole blood and plasma samples [23–25]. Impedance measurements were taken by adding a sinusoidal 10mV AC excitation signal across frequency up to 1 kHz. The resulting impedance spectra quantitatively illustrate the binding events at the electrode-electrolyte interface. IL-18 was tested over a concentration range of 170–717.2 pg/mL, and IL-10 was tested over a concentration range of 1–40 pg/mL, corresponding to systemic circulation levels. In Figs. 4A and B, the Nyquist analysis, the fundamental component of impedance plotted against the imaginary component of impedance (Z' vs. Z''), illustrates a clear trend as an increase in cytokine concentration results in varying changes in the semicircle diameter. This demonstrates the changes in the charge-transfer resistance upon antigen-antibody binding for both IL-18 and IL-10 in plasma, respectively. These shifts were consistent across the concentration ranges in plasma and confirm the electrochemical behavior across doses [26]. In Figs. 4C and D, the Bode analysis, the magnitude of impedance (Z_{mod}) plotted against a wide frequency range, also shows a clear dose-dependent response as the impedance magnitude increases proportionally with the cytokine concentration. Nyquist and Bode analyses revealed dose-dependent trends for IL-18 and IL-10 in plasma, consistent with the expected binding interactions at the electrode surface. This electrochemical confirmation highlights the sensitivity of the READ sensor in distinguishing incremental changes in pro- and anti-inflammatory biomarkers.

Correlation analysis and calibrated dose response

Correlation between matrices & pearson's correlation between potentiostats

Correlation analysis was done to evaluate the analytical performance of the READ platform. The READ platform was evaluated against a conventional benchtop potentiostat, and impedance values in plasma (Z_{mod} or magnitude of impedance) were compared. No significant differences were seen between the two systems, indicating that the handheld platform produces comparable impedance measurements to standard laboratory potentiostats (Figs. 5A, B). Due to the complexity of whole blood as a matrix in sensor detection, it shows higher variability, as illustrated by wider distributions in the box plots. Further correlation analysis was completed to evaluate the correlation between plasma and whole blood responses to validate the robustness of the system (Figs. 5C, D). Both IL-18 (right) and IL-10 (left) exhibited a strong linear correlation, with correlation coefficients (r) of

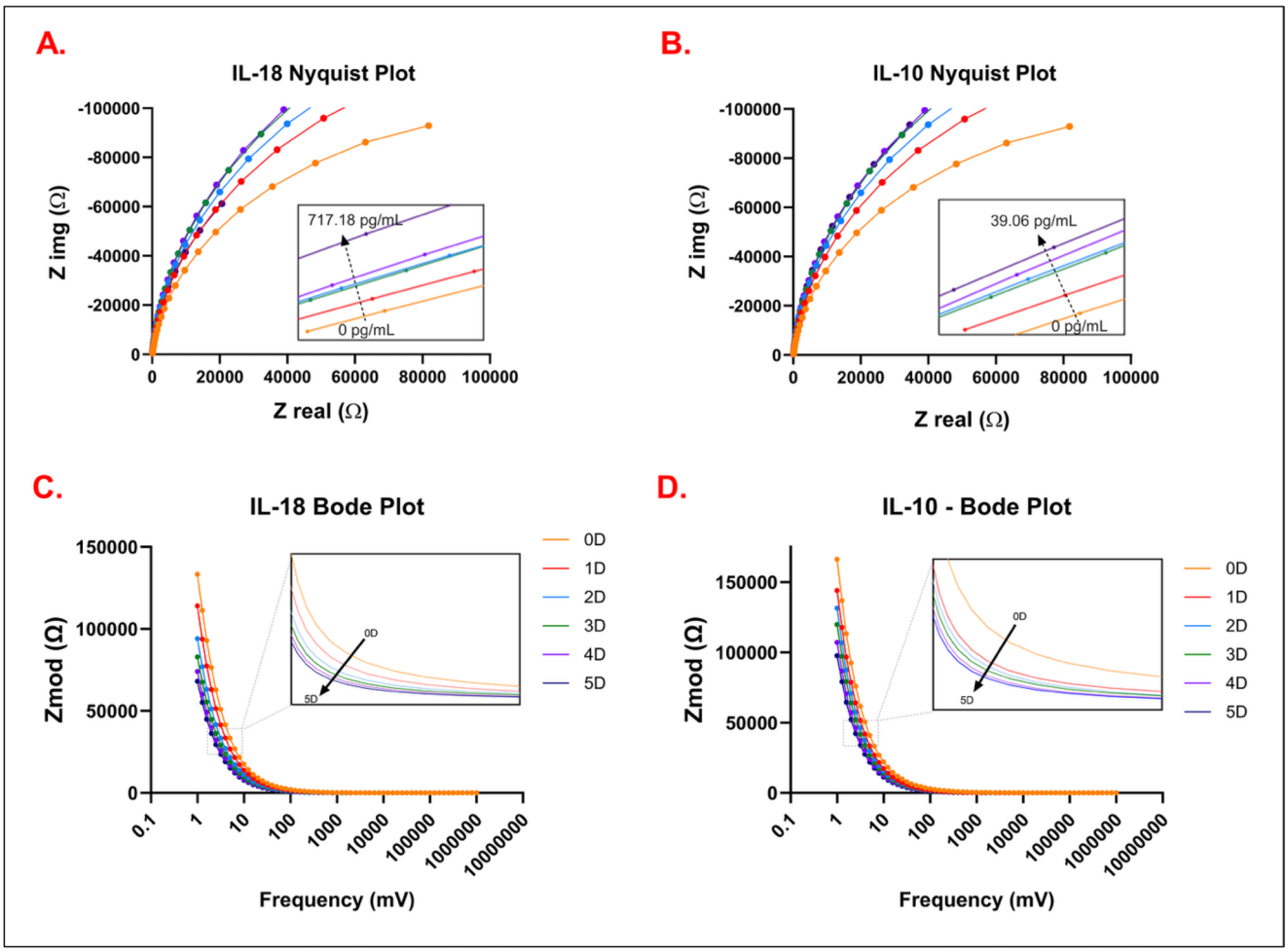

Fig. 4 Nyquist and Bode plots of IL-18 and IL-10 responses in plasma and whole blood. **A** Nyquist plot for IL-18, **B** Nyquist plot for IL-10, **C** Bode plot for IL-18, and **D** Bode plot for IL-10. Data shown correspond to impedance responses measured across increasing cytokine concentrations in plasma and whole blood

0.99 and coefficients of determination (R^2) exceeding 0.98, indicating that the platform maintains consistency across biological matrices. These results from the correlation studies confirm that the portable READ sensor achieves performance comparable to benchtop laboratory systems and is replicable across multiple bodily fluids.

Calibrated dose response study

Using the READ platform, impedance values across multiple replicates of electrodes were analyzed to generate a calibrated dose-response (CDR) curve, which correlates the impedance response with respect to the baseline (non-spiked samples of the matrix) to the incremental increases in antigen concentration (Figs.5E–H). Both cytokines exhibited concentration-dependent responses, as higher cytokine levels in the matrix yielded a higher change in the percentage response. In plasma, IL-18 achieved a regression coefficient (R^2) of 0.95 and IL-10 an R^2 of 0.93, reflecting strong reproducibility and linearity of the signal across the concentration ranges. Whole blood measurements also produced robust correlation, with R^2 values of 0.81 for IL-18 and 0.91 for IL-10. The complexity of the entire blood remains a factor here, as a more pronounced plateau effect was observed at higher concentrations. This is again likely due to the intrinsic complexity of whole blood, which can contribute to non-specific interactions and steric hindrance, thereby reducing the dynamic range of impedance changes relative to plasma. The CDR curves validate the sensitivity of the READ platform for the detection of pro- and anti-inflammatory cytokines in bodily fluids. There is consistent correlation in the calibration curves in plasma and whole blood matrices, which emphasizes the translational potential of the platform as a valuable tool for inflammatory monitoring and personalized disease management.

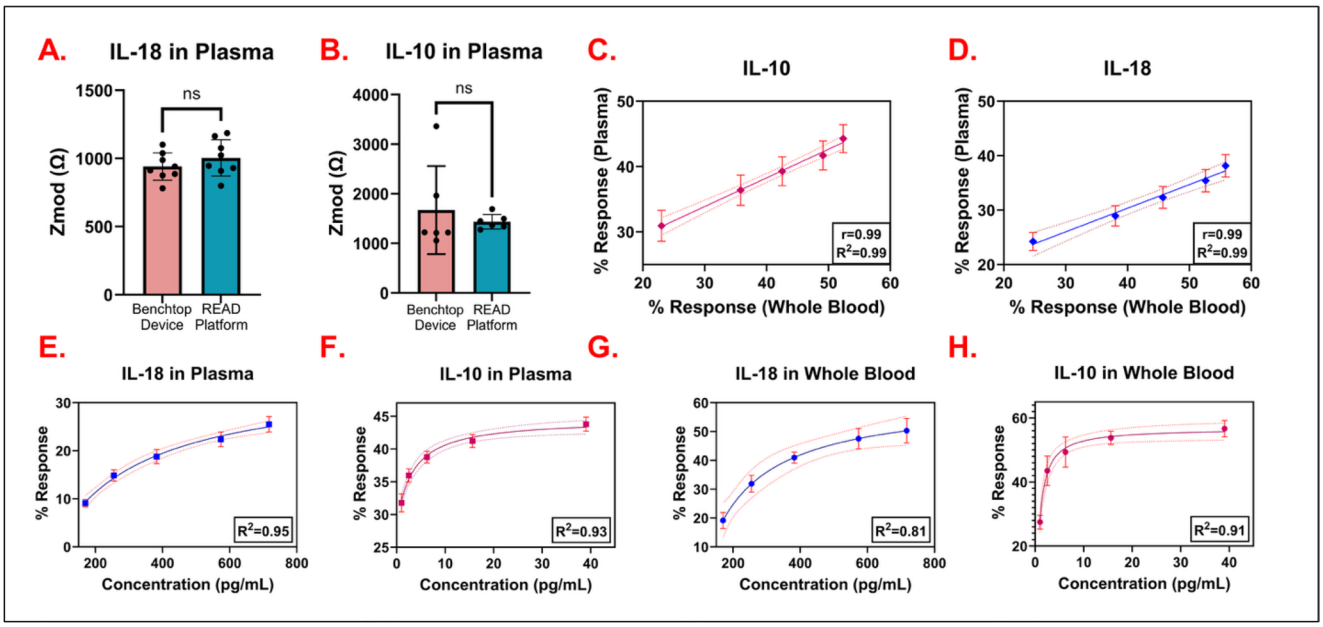

Fig. 5 (Top, left) Comparison of IL-18 **A** and IL-10 **B** % responses in plasma and whole blood, showing no significant (ns) differences between matrices.(Top, right) Pearson's correlation of % responses in plasma vs. whole blood for IL-18 **C** and IL-10 **D**, with linear regression analysis (R and R^2 values reported). (Bottom) Calibration dose–response (CDR) curves for IL-18 and IL-10 in plasma and whole blood **E**–**H**

Spike and recovery study

Spike-and-recovery performance

Spike and recovery experiments were completed to further evaluate the accuracy of the READ platform in recovering accurate concentrations from spiked samples of IL-18 and IL-10 in plasma and whole blood. Figures 6A–D shows the spiked versus recovered concentration plots, per marker per matrix, which demonstrated strong linear correlations, with R^2 values ranging from 0.96 to 0.99. Spike-and-recovery and corresponding % recovery and accuracy values are provided in Supplementary Tables 2 and 3, respectively. This analysis confirms the platform's ability to accurately measure cytokine concentrations in the proposed biofluid. To further assess sensor performance, percentage recovery and percentage accuracy were calculated (Figs. 6E–H). Both biomarkers per matrix fell within the clinical acceptance range between 80 and 120% (indicated by the dotted lines), underscoring the robustness of the assay. There was a broad linear response seen in plasma, whereas there was a slight plateauing of response in whole blood, likely due to matrix effects and consistent with the calibration curve. Protein interference and cellular content in whole blood may alter the response and reduce dynamic range; however, quantitative reliability is still demonstrated.

Specificity and cross-reactivity assessment

Specificity and cross-reactivity studies were performed to assess the selective response of the READ platform toward its target cytokines. Specificity studies were conducted by exposing the functionalized sensors (anti-IL-18 or anti-IL-10) to varying levels of the corresponding antigen to confirm an accurate target recognition response. Cross-reactivity studies were performed to evaluate the behavior of the sensor when exposed to different levels of non-target cytokines. Given the structural and functional stimulatory relationship between IL-18 and IL-10, our goal was to assess the platform's ability to discriminate between closely related analytes by using IL-18 as the non-specific marker during IL-10 testing, and vice versa. For each marker, non-specific low (NSL), specific low (SL), and their cocktail (SL+NSL) measurements were taken, as well as non-specific high (NSH), specific high (SH), and their cocktail (SH+NSH) measurements. In both plasma and whole blood, there was negligible signal contributions from non-specific and cocktail conditions, whereas distinct impedance shifts were seen in specific binding conditions (Fig. 6I-L). Cross-reactivity and specificity values are provided in Supplementary Table 4. These specificity and cross-reactivity studies confirm that the sensors exhibit high specificity and minimal cross-reactivity, allowing for proper differentiation between target and non-target cytokines across complex biofluids.

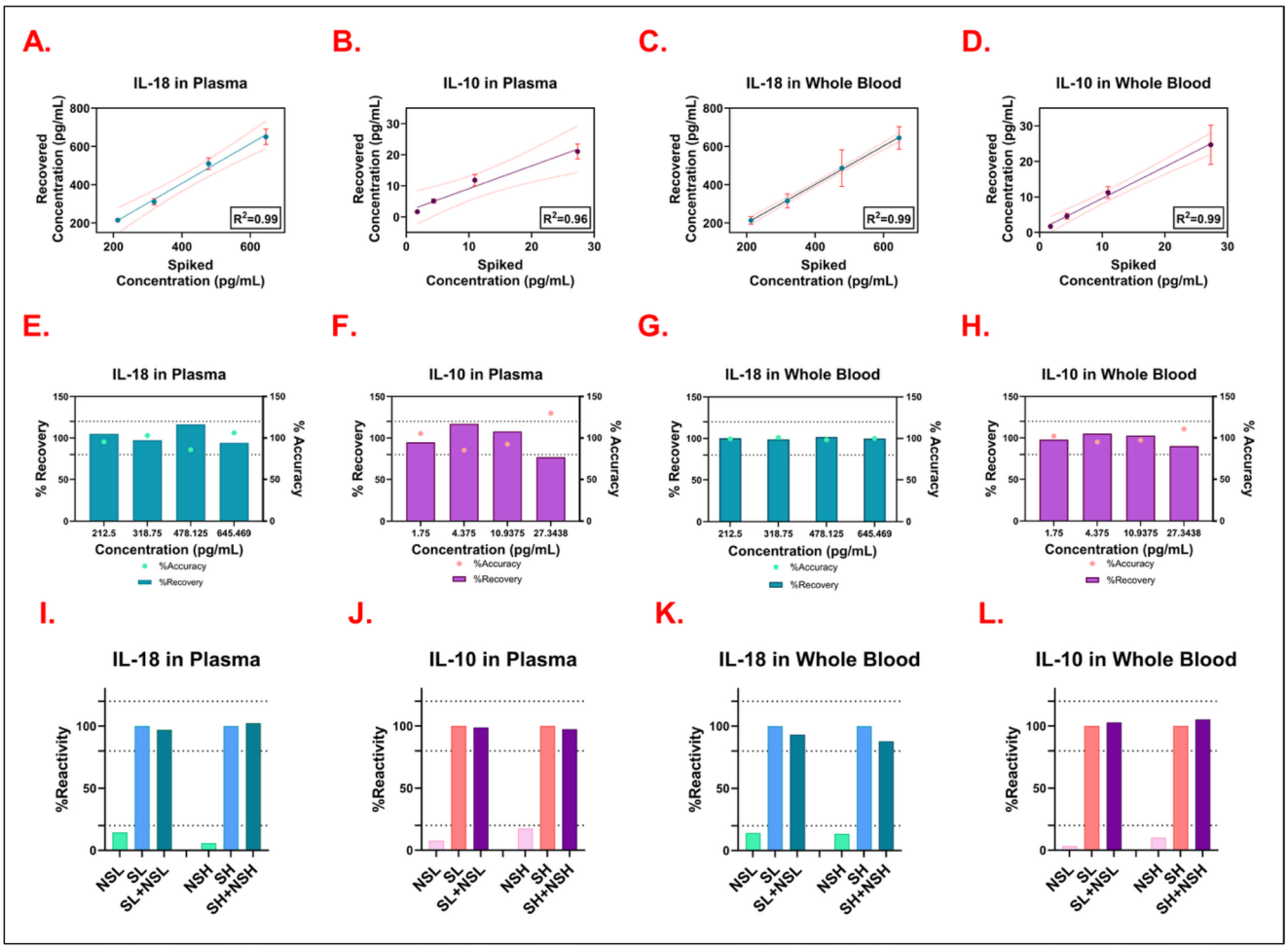

Fig. 6 Validation of READ sensor performance. **A–D** Spike-and-recovery (SR) plots showing spiked versus recovered concentrations of IL-18 and IL-10 in plasma and whole blood across clinically relevant ranges. Linear regression fits with R^2 values are indicated (mean ± SD, $n = 4$). **E–H** Percent accuracy and percent recovery analysis for IL-18 and IL-10 in plasma and whole blood. (I–L) Cross-reactivity assessment of IL-18 and IL-10 sensors in plasma and whole blood under low and high spike conditions. Non-specific low (NSL), specific low (SL), and their cocktail (SL + NSL) were tested, as well as non-specific high (NSH), specific high (SH), and their cocktail (SH + NSH)

Conclusion

This study successfully validates the efficacy of the READ sensor platform, a two-plex impedance-based (EIS) sensing platform, in simultaneously detecting interleukin-18 and interleukin-10 in blood-based matrices. IL-18 is elevated in multiple inflammatory and autoimmune states and is a valuable biomarker for disease stratification [27]. On the other end of the inflammatory response, IL-10 plays a key role in central immunomodulation as it holds prognostic value in systemic regulation, sepsis progression, and other states of dysregulated immunity [28, 29]. This dual-detection approach allows for an integrated view of the balance in the immune system as opposed to single-marker detection. Multiplex immuno-profiling is valuable in diagnosing dynamic disease states, as the overlap between pro- and anti-inflammatory signals can help catch inflammation before rapid disease progression.

The sensor chip-cartridge configuration requires less than 200 µL of sample volume to deliver quantitative results in under 10 min. It is well-suited for rapid, label-free point-of-care (POC) diagnostic purposes. The ZnO-modified gold sensor chip, functionalized by crosslinker-mediated antibody immobilization, demonstrated stable and specific sensor responses, resulting in robust signal transduction. The platform reliably quantified IL-18 over the dynamic range of 170 to 717.2 pg/mL and IL-10 over 1 to 40 pg/mL; both ranges encompassing the clinically relevant range in systemic inflammation. Both the calibrated-dose response and acceptable percent recovery and accuracy responses, per marker, confirmed the ability of the platform to detect the presence of and quantify the amount of cytokine available

in a patient sample. As a proof of concept, this study demonstrates the feasibility of simultaneously quantifying two immunologically distinct cytokines in blood-based matrices using the READ sensor platform. The portability of the device, paired with the minimal sample requirement and fast read-out, makes it a promising tool for decentralized monitoring of inflammation [30, 31].

Several advances can further improve the potential of the READ platform for use in translational medicine [29]. The platform establishes a foundation that can be broadened for future expansion towards multiplex detection of markers, diverse biofluids, and disease states to improve the immuno-profiling capability of the device [32]. The device can reach a higher potential by improving the long-term stability, storage longevity, and sensor variability between readings. The READ platform aims to incorporate the current assay development findings with these enhancements to evolve into a field-ready, multi-analyte biosensing device, capable of complex immune profiling and real-time patient monitoring.

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Declarations

Clinical trial number Not applicable.

Competing interests The authors declare the following financial interests/personal relationships, which may beconsidered as potential competing interests: Dr. Shalini Prasad has a significant interest in-Enlisense LLC, a company that may have a commercial interest in the results of this research andtechnology. The potential individual conflict of interest has been reviewed and managed by TheUniversity of Texas at Dallas, and played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report, or in the decision to submit the report forpublication. The portable device and technology platform are proprietary to EnLiSense LLC.

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