Tuning SLOCK toward Chronic Disease Diagnostics and Management: Label-free Sweat Interleukin-31 Detection

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ABSTRACT: SLOCK (sensor for circadian clock) is an electrochemical sweat-based biosensing platform designed for the diagnosis and management of circadian abnormalities. Previously, the SLOCK platform was designed to detect adrenal steroids, cortisol, and DHEA for tracking the circadian rhythm. This work aims at tuning this SLOCK platform toward the detection of the cytokine, interleukin-31, for building a noninvasive, chronic disease diagnostics and management platform. This research provides a detailed characterization of the sensing surface and immunochemistry. The results show that SLOCK has good sensitivity to IL-31 concentrations in synthetic and human sweat. The limit of detection is 50 and 100 pg/mL for synthetic and human sweat, respectively. The dynamic range of the system is 50–1000 pg/mL, which encompasses the physiological ranges of 150–620 pg/mL. This is the first demonstration of sweat-based, label-free, electrochemical detection of IL-31. In addition to this, the data show good correlation ($R^2 > 0.95$) for the signal sensitivity to biomarker concentration. Finally, cross-reactivity studies highlight the specificity of SLOCK even in the presence of highly cross-reactive species. Thus, this novel SLOCK biosensor can be successfully used to track IL-31 in a sensitive and noninvasive manner and could be used to identify chronic pathophysologies present in the body.

1. INTRODUCTION

The focus of healthcare technologies is shifting toward early detection, prevention, and management of chronic conditions. With this paradigm shift, there is an increased demand for patient-centric, noninvasive self-monitoring sensing platforms.1 Wearable sensing technology offers to be an attractive platform to facilitate continuous monitoring and self-management of health, especially for patients suffering from chronic disorders. With the advancements in technology, commercially available wearable sensors are becoming integrated into the user’s routine life. Some examples of these sensors include wearable health and activity monitors such as Apple watch, Fitbit, and Samsung galaxy watches. These devices continuously monitor various physiological activities such as heart rate and amount of physical activity. The next generation of wearables is designed to offer a comprehensive evaluation of the wearer’s physiological well-being (e.g., level of hormones, biomarkers, etc.). They are predicted to be employed for a plethora of applications ranging from healthcare-related applications such as health status monitoring, chronic disease management, and personalized medicine to non-healthcare applications such as athletics and nutritional wellness.2 The current global pandemic due to COVID-19 exacerbates the void in rapid and noninvasive diagnostic technology and calls for significant scientific progress in developing efficient wearable biosensing platforms.3,4

Sweat-based sensing offers a solution of providing a noninvasive and convenient source of health and disease profiling. Sweat composition has a significant number of biomarkers which have pathophysiological significance. Some examples include molecules such as cortisol and DHEA5,6 which are stress-related corticosteroids, molecules such as cytokines from the interleukin family, and molecules such as lactate and glucose,7 hormones and enzymes,8 and so forth. An important molecule, which is yet to be fully investigated but has relevance to disease diagnostics, is interleukin-31. Discovered in 2004,9 this biomarker comes from the glycoprotein 130 (gp130)/IL-6 cytokine family and has a molecular mass of 24 kDa in its glycosylated form.10 This is mainly expressed by Th2 CD4+ T cells (T helper cells) through signaling via a heterodimeric receptor complex.11 IL-31 is mainly involved in numerous pathways regulating immune responses, hematopoiesis, inflammation, and hypersensitivity. This biomarker is also involved in the etiopathogenesis of skin-based autoimmune disorders such as pruritis,
alopecia, skin lesions, and so forth. Moreover, it is overexpressed in patients suffering from atopic dermatitis as compared to patients with healthy skin. In addition to this, IL-31 levels were reported to increase in patients suffering from allergic rhinitis. Also, this biomarker was reported to increase under psoriatic skin conditions and airway hypersensitivities. With the involvement of IL-31 in disease progression or severity of chronic spontaneous urticaria, allergic contact dermatitis, prurigo nodularis, and mastocytosis, reports suggest using it as a potential diagnostic marker of allergic diseases. Furthermore, this molecule is suspected to be involved in carcinogenic pathways. The reason why IL-31 molecule is the biomarker of interest for this work is because of its recently discovered presence in sweat. Dai et al. reported that eccrine sweat contains IL-31, which is associated with skin inflammation and pruritus. It is expressed in the physiological range of 150–620 pg/mL. Thus, tracking IL-31 in sweat can provide a detailed outlook on the onset of symptoms and can be used for tracking disease progression.

This work looks at tuning the electrochemical detection capabilities of a sweat-based circadian diagnostic platform, SLOCK (sensor for circadian clock), for tracking a protein biomarker, IL-31. Previously, the SLOCK platform was used as a circadian monitoring system and was designed to detect cortisol and DHEA (steroid biomarkers). The work in this paper is a proof-of-feasibility that the SLOCK can be calibrated to perform protein biomarker detection for the ultimate purpose of transitioning to a chronic disease diagnostics and monitoring platform. The word tuning is used here to depict the process of calibrating and testing the sensitivity of the platform to the benchmarking protein of interest, IL-31. As discussed earlier, IL-31 is involved in pathways associated with various pathophysologies, specifically autoimmune and allergy-based disorders. This work aims at detecting IL-31 in sweat.
sweat using non-Faradaic electrochemical impedance spectroscopy (EIS) over the physiologically relevant range. Using non-Faradaic EIS ensures that the detection is label-free and a redox probe is not needed to capture the signal for binding. The platform is initially characterized using techniques such as scanning electron microscopy (SEM), EDAX, Fourier transform infrared (FTIR), surface plasmon resonance (SPR), and dynamic light scattering (DLS) studies. The SLOCK platform is then tested for its sensitivity in synthetic sweat (SS, with pH variation) and human sweat. A detailed analysis of the electrochemical signal for IL-31 concentration is provided with Randle’s circuit-based fitting. Finally, cross-reactivity studies with previously explored molecules such as cortisol and DHEA and similarly configured molecule such as IL-6 highlight the specificity of the SLOCK platform. Thus, the SLOCK biosensor can be successfully used to track IL-31 in a sensitive and noninvasive manner and could be used to identify chronic pathophysiology present in the body.

2. RESULTS AND DISCUSSION

2.1. Sensing Platform Characterization. 2.1.1. SEM and EDAX Studies. The sensing system uses a nanoporous polyamide (PA) substrate (pore size: 200 nm) as the base of the platform. Thin-film gold (150 nm) is deposited on the PA membrane, and this gold layer is used to functionalize the surface with a biorecognition probe for making the sensor specific to IL-31. SEM was used to highlight the cross-linked porous framework of the PA membrane, which is depicted in Figure 1a. This SEM micrograph highlights the porosity of the membrane and highlights the nucleation of the gold deposited on the fibers. A picture of the bare PA membrane (before gold deposition) is provided as Figure S1. This increases the surface area of interaction for increased capacitive response at the electrical double-layer (EDL) interface. In addition to this, nanoporous membranes contribute toward enhancing the signal by providing optimum interaction time by selective transport at the molecular level. Effective molecular entrapment combined with macromolecular crowding helps concentrate the biomarker near the interaction zones when the sample volumes are low. This is important and advantageous while working with platforms such as SLOCK that use passive sweating as their sweat sampling source. Passive sweating generates low volumes of samples as there is no stimulation of sweating as their sweat sampling source. The SLOCK platform works with ultralow volumes of 10 −150 glands, 15,16,17 the calculation predicts to generate a working sample between 6 and 10 min, without any additional efforts. This ensures that there is rapid and real time assessment of the sample.

Employing a nanoporous membrane also ensures that sweat containing the biomarker of interest wicks effectively through the substrate and allows for the maximum interaction between the target and the immobilized biorecognition element. Also, it helps with reducing the nonspecific response by decreasing the background noise contributed by the high-molecular-weight molecules present in sweat. Moreover, the macromolecular crowding enhances the biomolecular stability, specifically for proteins such as IL-31. 20

The EDAX spectrum highlights the elemental composition of the sensing platform and confirms the successful deposition of gold on the PA substrate. This is illustrated in Figure 1b and was performed in conjunction with SEM. The broad peak at 2.2 keV corresponds to the gold deposition over the PA membrane. The other peaks corresponding to the carbon, nitrogen, and oxygen are a function of the elemental composition of the PA membrane. Thus, the EDAX spectrum confirms the successful deposition of gold electrodes on the nanoporous polyamide substrate. The gold-deposited PA is then used as a substrate to facilitate anchoring of the IL-31-specific biorecognition probe on the sensor surface.

2.1.2. FTIR Study for Immunochemistry Characterization. FTIR spectroscopy is used to characterize the immunochromistry responsible for imparting specificity to the sensing platform. The biorecognition probe in this sensing platform’s case is a monoclonal antibody that is designed to be specific for the IL-31 protein molecule. The FTIR spectrum was collected on the antibody-immobilized gold PA membrane and compared with the control, which was the bare gold PA membrane. This is illustrated in Figure 1c. Table 1 provides a summary of these peaks along with the molecule of interest. The thiol linker is used to activate the gold surface and help with the anchoring of the antibody onto it. The thiol-gold affinity can be seen by the appearance of the stretching of CH alkane observed between 3400 and 3000 cm −1 and between 2850 and 2960 cm −1 wavenumber range. The thiol linker binds to the gold on one end and has an amine-reactive NHS ester on the other end. When the antibody is introduced on the linker-immobilized surface, the ester reacts with the primary amines of the antibody to form amide bonds. This reaction ensures the chemisorptive anchoring of the antibody on the sensing surface. From the FTIR spectrum, antibody immobilization is evident from the peaks between the 1600−1655 cm −1 range for the amide I bond and the 1500−1580 cm −1 for the amide II bond. 19,21 These peaks are contributed by the secondary structure of the protein molecule, that is, antibody. Also, the appearance of the CNC stretch of the aliphatic amines in the range between 1000 and 1200 cm −1 highlights the successful conjugation between the thiol linker and the antibody. This confirms the successful immobilization of the biorecognition probe when compared to the bare PA spectrum. In addition to this, the peak between 1050 and 1080 cm −1 corresponds to the C−O bond due to the amide band in proteins. 22 Thus, the FTIR spectrum confirms successful immobilization of the immunochromistry responsible for the detection of IL-31 in sweat.

2.1.3. Hydrodynamic Radius and ζ Potential Studies. DLS was performed to characterize the binding interactions between the IL-31 antibody and antigen. Understanding how the charge and electrophoretic behavior changes between the
bound and unbound stages of the proteins is crucial to developing an affinity-based detection system. The principle of working revolves around the mobility and diffusivity of the molecules undergoing Brownian motion while suspended in a solution. The charge and stability of the compound determine the properties of the EDL formed around the protein surface. In this work, we evaluated the change in hydrodynamic radius for binding between the IL-31 antibody, low dose (50 pg/mL) and high dose (500 pg/mL) of IL-31 antigen. From Figure 1d, it can be observed that the radius increases from $13.2 \pm 0.2$ to $17 \pm 0.8$ with the binding caused by the introduction of the IL-31 molecule in the system. However, going from lower to higher dose, there is no significant change in the size of the complex formed. This highlights the preservation of the conformational state of the protein complex, thus ensuring stability.

Furthermore, analyzing the $\zeta$ potential helps with understanding the charged states and the electrochemical nature behind the binding. The sensing platform uses an electrochemical detection modality for characterization of the system’s sensitivity to the concentration of the target biomarker. For this reason, evaluating the $\zeta$ potential helps with understanding of the binding in the presence of an EDL. The results from the $\zeta$ potential study are depicted in Figure 1d. The $\zeta$ potential changes from $-15.1 \pm 0.24$ to $-13 \pm 0.36$ with the addition of the IL-31 biomarker. With the increase in dose concentration from a low dose (50 pg/mL) to a high dose (500 pg/mL), there is a significant change in the $\zeta$ potential (**$p < 0.01$). This is due to the binding between the antibody and antigen to form a complex with higher surface charge around it. This higher surface charge has the ability to repel against similar charges within the sample, which leads to higher electrophoretic mobility. This higher electrophoretic mobility leads to an increase in the $\zeta$ potential, as observed from Figure 1d. Thus, from these studies, it can be concluded that the antibody is successfully immobilized on the sensing surface and is capable of sensitively detecting the IL-31 antigen.

Figure 2. SPR characterization of IL-31: (a) SPR signal for sensor functionalization and characterization of the IL-31 antibody and (b) kinetic model fit for $K_D$ evaluation of the IL-31 antibody.
2.1.4. SPR Studies. SPR was used to characterize the IL-31 antibody and the binding kinetics using a protein A molecule. SPR is an optical technique for understanding the molecular interactions in the sample. The electrode consists of a metal coated with carboxyl chemistry, which is then functionalized with a protein A assay and IgG antibody. The steps for the assay along with their signal responses are presented in Figure 2a. The principle of SPR is that when there is binding between the immobilized molecule and the ligand, there is a change in the local refractive index that changes the SPR angle. This SPR angle is monitored in real time by measuring the intensity of reflected light using a sensogram. For understanding the binding kinetics, the rate of change in this SPR angle is evaluated.25 In the FTIR studies, the sensor chemistry for performing IL-31 detection was performed. This section looks at the activity and binding of the antibody specifically. The protein A molecule is highly specific for the Fc region of the antibody and is used to anchor the antibody in the upward position. This ensures that the antigen-binding sites are exposed and are easily available for the antigen to bind. It is important to understand if the antibody has the ability to bind and produce a significant signal. Figure 2a highlights the process of IL-31 immobilization on the SPR carboxyl sensor chip. The protocol is described in detail in the Materials and Methods section. The antibody is immobilized in channel 2 of the microfluidic SPR cell in order to understand the level of binding against the control. It is evident from the graph that the signal is significantly higher for the IL-31 antibody as compared to channel 1 (control). This confirms that the antibody is active as the signal is between 1000 and 2000 RU (response units). The IL-31 antibody in the SLOCK sweat-sensing system is used as a biorecognition probe. For performing detection in sweat, the IL-31 probe needs to be sensitive enough so that it can detect low (picogram) ranges of the biomarker in the sweat sample. This was evaluated by performing the kinetic evaluation of the antibody molecule. A 1:1 binding model was used to perform the fitting for the antibody molecule, and the $K_D$ value obtained was $9.12 \times 10^{-11}$ M. Affinity is inversely proportional to the $K_D$ value, and the result highlights that the IL-31 antibody has high affinity and is able to functionalize on the surface with good sensitivity.26 High affinity antibodies have $K_D$ values on the range of low nanomolar ($10^{-9}$) to picomolar ($10^{-12}$) range.27 Thus, the IL-31 antibody can be used as a sensitive biorecognition element for performing detection using the SLOCK platform.

2.2. Electrochemical Sensor Response for Biomarker IL-31. Electrochemical sensing is the modality of choice for wearable sensing platforms. This is due to advantages such as rapid output, wider ranges of operation, high sensitivity and...
specificity, easy coupling to portable electronics, and portability.\textsuperscript{28,29} Non-Faradaic impedimetric sensing specifically has the advantage of making the response label-free and highly sensitive to low concentrations of biomarkers.\textsuperscript{30} In this work, we use non-Faradaic EIS for characterizing the sensing platform’s sensitivity to the concentration of IL-31 present in the system. Previously, the SLOCK platform was tested for its sensitivity to circadian-relevant biomarkers: cortisol and DHEA.\textsuperscript{16,17} These biomolecules are adrenal steroids present in sweat in the ng/mL range. While tuning the platform to perform the detection of a protein biomarker, it is imperative that the platform is able to provide the same sensitivity for a lower concentration range, as these molecules are often secreted in the low picogram range. Similar to the previously published work, this platform also uses ultralow volumes of sweat samples (10 μL), thus enabling the use of passive sweat sampling.

The sensing response was tested in a SS formulation that mimics the electrochemically active composition of the human sweat matrix. The sensor was tested in SS with pH variation in order to evaluate the stability of the sensing response in the presence of pH fluctuations. Sweat pH is reported to fluctuate within the range of 4–8, due to various factors such as amount of physical activity, diet, amount of sleep received, and so forth.\textsuperscript{6} Thus, the platform was tested in SS formulation of pH’s 4, 6, and 8, with pH 6 being the physiologically stable pH. The sensor response is displayed in Figure 3a. The signal response was tested against dose concentrations spanning 50–1000 pg/mL range. The physiologically relevant range spans from 150 to 620 pg/mL.\textsuperscript{15,31} The signal response for concentration dependency is illustrated in red for pH 4, blue for pH 6, and green for pH 8. From the figure, it can be observed that the response linearly increases with the increase in dose concentration. For pH 4, the sensor is able to differentiate between the low and high concentrations of the biomarker with good significance (\(p < 0.05\)). For pH 6, the sensor is able to differentiate between the low and high biomarker levels with high significance (\(p < 0.05\)). For pH 8, the sensor is able to differentiate between the low, medium, and high biomarker levels with high significance (\(p < 0.05, * * p < 0.01, \text{and} * * * p < 0.001\)). The limit of detection (LOD) for the SS response is 50 pg/mL. This is evident from the specific signal threshold (SST) (dotted line in Figure 3a) depicting the signal-noise threshold. The dynamic range of operation is 50–1000 pg/mL. An in-depth analysis into the electrochemical response can be provided by fitting the response using a modified Randle’s circuit. This Randle’s circuit, as depicted in Figure S2, consists of \(R_s\) (solution resistance), \(R_t\) (charge-transfer resistance), \(C_{\text{edl}}\) (capacitance of EDL), and \(C_w\) (Warburg resistance). The Warburg element is contributed by the diffusion and is evident at very low frequencies. The three main components responsible for contributing to the electrochemical signal response are \(R_s, C_{\text{edl}}\), and \(R_t\). The biomarker concentration-dependent modulation of these elements is presented as Figure 3b–d. For all the three pH’s, the components \(C_{\text{edl}}\) and \(R_t\) increase with the increase in dose concentrations. Whenever there is binding between the antigen IL-31 and the immobilized antibody, there is charge rearrangement giving rise to capacitative modulations in the EDL. These capacitive modulations are reflective of the increase in the \(C_{\text{edl}}\) component (blue curve) for the CDR response in SS pH 4, 6, and 8. This EDL is situated at the electrode–electrolyte interface which can be tapped into by analyzing the electrochemical response at the low 1–1000 Hz frequency range.\textsuperscript{32−34} Thus, the operating frequency was chosen as 15 Hz, where the maximum capacitive behavior of the signal was observed. This maximum capacitive behavior is evident as the phase shifts near −90° in the Bode phase plot, presented in the next section. The amount of charge-transfer resistance also increases with the amount of biomarker added because the antigen is a protein target. With the amount of protein biomarker being added to the system and binding to the capture probe, these insulative molecule introduces a resistance to the transfer of charges. This is evident by the increase in \(R_t\) (red curve) for the CDRs in SS pH 4, 6, and 8. A combination of both the \(R_s\) and \(C_{\text{edl}}\) response highlights the sensitivity of the platform to biomarker concentration. The range for fitting of \(R_t\) for SS pH 8 has a lower dynamic range, and there is a dominant capacitive effect observed. However, by observing the impedance response of the sensor in SS pH 8, it maintains the same dynamic sensitivity over the physiological concentration of IL-31. Thus, it can be inferred that SS pH 8 response is capacitive-dominant as compared to SS pH 4 and 6. Also, the solution resistance, depicted by the black curve, is contributed by the bulk solution effects. Ideally, the signal response should be minimally affected by the bulk solution effects. While working with highly ionic solutions such as human sweat, the platform needs to be robust enough so that it does not get affected by bulk effects of the buffer. This also ensures that the sensor’s specificity is high and that the change in signal is caused solely due to binding. This effect of bulk solution can be computed by looking at the trend of the \(R_t\) component of the Randle’s circuit fitting result.\textsuperscript{23} From Figure 3b–d, it can be observed that the \(R_t\) lies in the lower 0–400 Ω range and does not show any trend with the addition of new doses. Overall, it can be concluded that the signal is not affected by the bulk effects of the solution and that the electrochemical sensing response is contributed mainly from the binding phenomenon at the EDL layer. Thus, the SLOCK sensing platform has the ability to respond sensitively to the IL-31 concentrations and has low noise contributed by the background ions present in the sample.

Furthermore, a clear dose-dependent response is observed in the Nyquist plots for the CDR in SS. This is presented in Figure S3. Nyquist plots are the frequency signatures of the impedance response. They show the variation between the imaginary versus the real component of the impedance over the course of the entire frequency range (in this case, 1–10 kHz). The Nyquist plot of a non-Faradaic response is characterized by a continuous incomplete semicircle, as seen in Figure S3. From the figures, it can be observed that with the increase in dose concentration, the radius of curvature of the impedance curve increase. This validates the electrochemical fitting data that were described in the previous paragraph, where the radius of curvature of the impedance corresponds to the \(R_t\) of the system. In addition to this, the offset of the curve on the x-axis highlights the \(R_t\) contribution to the signal. From the Nyquist plots, it can be observed that the amount of \(R_t\) modulation does not significantly vary with dose concentrations and do not impact the sensing response. In addition to this, there is a clear trend observed for biomarker sensitivity across the three pH’s. This highlights that the sensing platform is able to retain its sensitivity and selectivity for IL-31 in the presence of pH fluctuations. The next section investigates the sensitivity of SLOCK to IL-31 levels in a real biofluid matrix.
2.3. Dose Response Study of IL-31 in Human Sweat.
There is a significant amount of difference between an artificial matrix and a real biological matrix. In order to understand the true sensitivity of the platform to IL-31 concentrations, it was tested in human sweat and the impedance response was extracted. This is presented in Figure 4a. The CDR is highlighted by the graph, where the green section is the normal physiologically relevant range of IL-31. The black dotted line signifies the noise-signal threshold or the SST. This is calculated by the blank dose of human sweat without any IL-31 present in it. The platform shows incremental change in sensing response to the increase in IL-31 concentrations. The platform can sensitively distinguish between the normal and elevated levels of the biomarker in human sweat (**p < 0.01).

Apart from having a high amount of ionic moieties present, sweat also contains proteins, hormones, enzymes, and so forth present in the mix. The ratio change in impedance highlights the change in the signal from a baseline which was the blank human sweat dose. By calculating the change, the basal levels of the biomarker present in the human sweat sample can be accounted and adjusted for. This ensures that the output signal corresponds to the biomarker concentration it is being tested with. The data show good correlation (R² > 0.95) for the signal and biomarker concentration. The LOD for the human sweat response is 100 pg/mL, and the dynamic range is 100–1000 pg/mL. In addition to this, the impedance response plots, Nyquist and Bode, reinforce the sensitivity of the SLOCK platform to the biomarker concentration. This is illustrated in Figure 4b,c. The Nyquist plot highlights the dose-dependent trend from baseline (0 pg/mL) to 1000 pg/mL. Similar to the Nyquist plot for the SS formulation, the human sweat shows an increase in R<sub>c</sub> with the increase in IL-31 concentration with minimal effects of R<sub>s</sub> observed. Thus, despite being a complex solution as compared to the synthetic formulation, the SLOCK platform is able to detect IL-31 in human sweat with good sensitivity. The Bode plot highlights the clear signal dependence on the frequency going from the surface of the sensor (higher frequency range 1000–10 kHz) to the electrode–electrolyte interface (low frequency range 1–1000 Hz). The Bode plot presented in Figure 4c shows the capacitive nature of the signal and confirms the consistent use of 15 Hz operational frequency for the maximum capacitive response. As compared to the synthetic formulation, the Nyquist plot shows a wider dynamic range across the range of IL-31 concentration. This wider dynamic range of the curve indicates that the sensing platform is able to sensitively respond to each change in the concentration of IL-31. In addition to this, these results highlight the ability of tuning SLOCK, a circadian sensing platform, to perform IL-31 detection in the physiologically relevant ranges. This provides the proof-of-feasibility of the platform to be eventually used as a chronic disease diagnosis and management platform. Also, the results clear the path of adopting the sensing platform as an on-body sweat-based self-monitoring device.

2.4. Reproducibility and Repeatability. The performance of SLOCK for the detection of IL-31 highlights its ability of capturing the secretion of a protein-based biomarker in passive sweat. In order to understand the true response of the
system, inter- and intra-assay variability was evaluated in the SS (pH 6) and human sweat platform. The interassay reproducibility is presented in Figure 5a, b, and the intra-assay repeatability is presented in Figure 5c, d. The coefficient of variation (% CV) for IL-31 concentrations from N = 3 sensors is plotted in Figure 5a for SS and Figure 5b for human sweat. The sensor exhibits a CV < 20% for the IL-31 assay across the different concentrations. This is acceptable as per the guidelines set for interassay variability by the Clinical and Laboratory Standards Institute (CLSI). Similarly, the intra-assay variation is illustrated in Figure 5c, d for SS and human sweat, respectively. This was performed over n = 5 cycles. Across the IL-31 biomarker concentrations, the intra-assay variability stays under 5%. This variability highlights the repeatability of the performance within the assay. These results are also CLSI guideline compliant (<10% intra-assay variation). It can be inferred from these results that the performance of SLOCK for detecting IL-31 in a sweat matrix is reproducible and repeatable.

2.5. Recovery and Cross-Reactivity Study. Recovery studies were carried out to understand the sensor performance with respect to the standard dose response curves (CDR). The SLOCK platform was spiked with three different concentrations (50, 250, and 500 pg/mL) to calculate the percentage recovery. From Figure 6a, it can be observed that the SLOCK platform shows good recovery (>95%) for low (50 pg/mL), medium (250 pg/mL), and high (500 pg/mL) dose concentrations of the antigen molecule. This recovery is acceptable according to analytical standards and clearly demonstrates the sensitivity of the platform to IL-31 concentrations in the sweat matrix.

The SLOCK platform was tested for the specificity to IL-31 by comparing the cross-reactive response with commonly found biomarkers in sweat. An artificial cross-reactive solution with a high concentration of molecules such as cortisol, DHEA, and IL-6 was created in SS pH 6. The SLOCK platform was previously tested for the sensitivity to cortisol and DHEA.6,16 In this work, the SLOCK platform was immobilized using an IL-31-specific capture probe, so it is imperative that the sensor does not produce a significant signal for the cortisol and DHEA molecules. Also, in addition to this, IL-6, another proinflammatory cytokine with a similar structure to IL-31, was included in the cross-reactive solution. The IL-31 molecule hails from the four α-helix bundle family and is related to the IL-6 subgroup. Thus, there is a high probability of competition for the target between the two interleukin molecules. This competition can result in false elevation of the signal which is not specific for IL-31. The result from the cross-reactivity study is presented in Figure 6b. The blue bar represents the specific response for 1 ng/mL (highest physiological concentration) of IL-31 and the red bar represents the signal from the cross-reactive solution consisting of 10 ng/mL concentration of cortisol, DHEA, and IL-6 each. It can be observed that the signal for IL-31 is significantly lower compared to the cross-reactive molecules.
3. MATERIALS AND METHODS

3.1. Reagents and Materials. The thiol linker used for anchoring capture probe 3,3-dithiobis(sulfosuccinimidyl propionate) (DTSSP) and phosphate-buffered saline (PBS) was procured from Thermo Fisher Scientific Inc. (Waltham, MA, USA). The IL-31 antibody (capture probe) was also purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). The IL-31 antigen (protein) was purchased from Abcam (Cambridge, MA, USA). SS was prepared according to previously published protocols. Human sweat was purchased from Lee Biosolutions Inc. (Medix Biochemica Group, MO, USA).

3.2. Sensor Fabrication and Immunoassay. Sensors were fabricated in the cleanroom facility at the University of Texas at Dallas. Gold was deposited on the nanoporous PA membrane (GE Healthcare Lifesciences, NJ, USA) using a shadow mask in a cryo e-beam evaporator. The cryo e-beam evaporator was chosen to perform thin-film deposition of gold (150 nm at 1 Å/s) on the PA surface because of its tool accuracy and minimum batch–batch variation. This tool is housed in the cleanroom facility at the University of Texas at Dallas. The deposition has been confirmed with profilometry data presented in Figure S4. Detailed fabrication description has been provided in the previous work. Sensors were functionalized using a solution of DTSSP and IL-31 antibody (10 μg/mL) for a period of 24 h at 4 °C.

3.3. Experimental Details for FTIR Studies. FTIR spectroscopy was carried out using the Thermoscientific Nicolet iS50 FTIR machine in the attenuated total reflectance mode. The sample was prepared on the gold-deposited PA membrane and compared with the blank gold-deposited PA membrane. The spectrum was recorded between 800 and 4000 cm⁻¹ with a resolution of 0.5 cm⁻¹ and 256 scans.

3.4. Experimental Details for Hydrodynamic Radius and ζ Potential Studies. Hydrodynamic radius and ζ potential was measured using Malvern Zetasizer NanoZS (Malvern Instruments, UK). The hydrodynamic radius was recorded for antibody in PBS, followed by a low dose of biomarker (50 pg/mL) and a high dose of biomarker (500 pg/mL). ζ potential was calculated from the electrophoretic mobility using the Smoluchowski equation.

3.5. Experimental Details for SPR Studies. SPR characterization studies were performed using the OpenSPR instrument from Nicoya (Canada). A protein A immobilization kit along with a carboxyl chip was employed for characterization of the IgG IL-31 antibody. The protocol involved surface cleaning with 10 mM HCl (pH 2–3), followed by EDC-NHS immobilization. This was followed by loading of protein A molecule (20 μg/mL) and 50 μg/mL of antibody solution (Thermo Fisher Scientific Inc.).

3.6. Experimental Details for EIS Studies. All electrochemical studies were performed using a Gamry Reference 6000 potentiostat (Gamry Instruments, PA, USA). The EIS setup was similar to the one described in the previous publication. The IL-31 biomarker concentrations were spiked in SS and human sweat and were drop-casted on the sensor surface. The impedance response for each change in concentration was recorded and extracted at 15 Hz (maximum capacitive behavior). This is presented as ratio change in impedance from the blank dose (zero dose). Optimized incubation times were used for dosing intervals. The range of doses spanned from 50 to 1000 pg/mL, which covered the physiological range of 150–620 pg/mL in sweat.

3.7. Statistical Analysis. The data presented are plotted as mean ± SEM. The data are presented as N = 3 and interassay and intra-assay variation is <10% (CLSI guideline compliant). Unpaired t-test and ANOVA with post hoc Tukey test were performed to calculate significance. All the statistical studies were performed using GraphPad Prism version 8.01 (GraphPad Software, La Jolla, CA, USA).

4. CONCLUSIONS
This work aims at tuning SLOCK, a sweat-based, circadian-relevant biomarker-sensing platform, to perform the detection of IL-31. IL-31 can be used to identify chronic pathophysiology present in the body and is expressed in sweat in the range of 150–620 pg/mL. This work uses EIS to evaluate the sensitivity of the platform. The LOD is 50 and 100 pg/mL for SS and human sweat, respectively. The dynamic range of the
The system is 50–1000 pg/mL, which encompasses the physiologically relevant ranges of 150–620 pg/mL. In addition to this, the data show good correlation ($R^2 > 0.95$) for the signal sensitivity and biomarker concentration. Furthermore, a detailed analysis of the electrochemical response is provided by fitting studies using a modified Randle’s circuit. Furthermore, in- and interassay variability (% CV) is under 10%, highlighting that the performance is reproducible and repeatable. Finally, cross-reactivity studies highlight the specificity of the platform for the detection of IL-31. Thus, the SLOCK platform was successfully tuned to detect IL-31 in sweat using ultralow volumes of the sample. This platform is envisioned to serve as a vessel for performing self-monitoring of chronic conditions and help with better prognosis.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/10.1021/acsomega.1c02414.

Randle’s equivalent circuit, SEM images, Nyquist plots, and profilometry data for the sensor data in SS (PDF)

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## Author Contributions

Experimental data collection and curation; methodology; formal analysis; resources and software; and writing—original draft, review, and editing—S.U., conceptualization; investigation; methodology; visualization; supervision; and writing—review and editing—S.P. All authors have given approval to the final version of the manuscript.

## Notes

The authors declare the following competing financial interest(s): Dr. Shalini Prasad has a significant interest in Enlisense LLC, a company that may have a commercial interest in the results of this research and technology. The potential individual conflict of interest has been reviewed and managed by The University of Texas at Dallas and played no role in the study de-sign; in the collection, analysis, and interpretation of data; in the writing of the report, or in the decision to submit the re-port for publication.

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## ABBREVIATIONS

SLOCK, sensor for circadian clock; IL-31, interleukin-31

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