Electrochemical stability of PEDOT for wearable on-skin application

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Abstract
Conducting polymers are promising candidates for wearable devices due to mechanical flexibility combined with electroactivity. While electrochemical measurements have been adopted as a central transduction method in many on-skin sensors, less studied is the stability of the active materials (in particular poly3,4-ethylenedioxythiophene, PEDOT) in such systems, particularly for “on-skin” applications. In this study, several different variants of doped PEDOT are fabricated and characterized in terms of their (electrical, physical, and chemical) stability in biological fluid. PEDOT doped with tosylate (TOS) or polystyrenesulfonate (PSS) are selected as prototypical forms of conducting polymers. These are compared with a new variant of PEDOT co-doped with both TOS and PSS. Artificial interstitial fluid (aISF) loaded with 1% wt/vol bovine serum albumin is adopted as the testing medium to demonstrate the stability in dermal applications (i.e., conducting polymer microneedles or coatings on microneedles). A range of techniques such as cyclic voltammetry and electrochemical impedance spectroscopy are used to qualify and quantify the stability of the doped conducting polymers. Furthermore, this study is extended by using human skin lysate in the aISF to demonstrate proof-of-concept for stable use of PEDOT in wearable “on-skin” electronics.

KEYWORDS
biomedical applications, conducting polymers, electrochemistry

1 | INTRODUCTION
The concept of organic conductors/semiconductors has arisen since the discovery of highly conducting polyacetylene in 1977. Since then, much effort has been placed on researching these polymers, encouraged by their versatility due to biocompatibility, chemical stability, high processability, low-production cost, and being electrically tunable. Rapid growth has ensued in research focused on conducting polymers in biomedical engineering areas such as synthetic neural networks, artificial muscles, and engineered tissue. Recent works have also highlighted integration of conducting polymers in wearable and skin-attachable biomedical devices.
This is stimulated by the embodied properties of polymers and inorganic semiconductors within the conducting polymer such as lightweight, ease of process and mechanically flexible combined with electrically conductive for soft electronics.\textsuperscript{1,7,11} Recent demand of on-skin, wearable sensing technology is shifting to noninvasive diagnostic and therapeutic technologies, which are driven by sensing of a biomarker or drug from sweat\textsuperscript{12} or interstitial fluid (ISF)\textsuperscript{13}—the thin layer of fluid under the skin epidermis layer. The development of conducting polymers in noninvasive wearable sensors have been reported for sensing of lactate,\textsuperscript{14} calcium ion,\textsuperscript{15} sodium ion,\textsuperscript{16} ammonium,\textsuperscript{10} where sweat is the sensing medium.

To their composition, ISF and blood presumably give more accurate information about a person’s body chemistry as compared to sweat.\textsuperscript{13,17} In the monitoring of glucose levels in diabetic patients, the ISF has been shown to possess similar information to blood.\textsuperscript{18,19} From the perspective of user comfort, there is a need to sample these bodily fluids from under the skin without causing much pain. Microneedles are one way in which to sample the ISF in a noninvasive manner.\textsuperscript{20} Bollella et al. recently integrated minimally invasive microneedles with in situ electrochemical biosensing within dermal ISF.\textsuperscript{21} While several similar studies have reported continuous glucose monitoring where the microneedle not only acted as the skin-puncture device, but also the electrodes for electrochemical measurements.\textsuperscript{19,22} Further development of the microneedles have utilized Au\textsuperscript{19} and Pt\textsuperscript{22} deposited on the microneedle surface for better sensitivity of glucose sensing. Given this shifting focus to sensing in ISF, the studies herein will give insight to the potential of conducting polymers in wearable devices, such as coatings on microneedles or the microneedle structure itself, focusing on the behavior in artificial ISF (aISF).

The most conventional analysis system for on-skin sensing is via electrochemical testing, due to high selectivity and sensitivity to analytes, low-device cost, and fast response of the sensor.\textsuperscript{23} Central to building an electrochemical sensor is the (electro) active material used to specifically detect and quantify the biomarker of interest in the sweat and ISF. Conducting polymers have also been explored as electroactive materials in electrochemical biosensors\textsuperscript{24} due to high-chemical stability, electrochemical activity, and electron mobility, which resulted in better facilitation of electron transfer in the system. Though many papers have reported conducting polymers in electrochemical sensing,\textsuperscript{25} only a few studies have been published on the polymer’s stability in the electrochemical system.\textsuperscript{26,27} To our knowledge, no publication has discussed PEDOT’s electrochemical stability in aISF. As preparation for on-skin application focusing on sensing within the ISF, the simulated biological fluid tested in the electrochemical system will be aISF + bovine serum albumin (BSA), and aISF combined with human skin lysate. BSA is a common serum protein, which have been employed in previous works as to test performance of material in biological environment.\textsuperscript{28} In this work, the addition of BSA is to provide biological constituents, that is, proteins to the aISF to observe whether these proteins may interfere with performance of PEDOT. The use of the human skin lysate is critical in demonstrating the suitability of the PEDOT materials for wearable “on-skin” applications due to assumed similarity of the biological constituents of the human skin lysate with additional constituents of the biological fluid within the skin.

With respect to conducting polymers, counterions, or dopants are added to their structure to increase the number and mobility of charge carriers within the polymer’s π-bonded network.\textsuperscript{29} These counterions stabilize the charge carriers (most often positive charges/holes) such that they can move along individual polymer chains and/or hop between neighboring chains. While PEDOT performance is heavily affected by the dopants, this study explores effects of two types of dopants, that is, molecular dopant, represented by tosylate (TOS) and polymeric dopants, represented by polystyrenesulfate (PSS). A new PEDOT variant with hybrid dopants, by combining both TOS and PSS into PEDOT is also included in the studies. In general, the three types of PEDOT are PEDOT:TOS, PEDOT:PSS, and PEDOT:TOS/PSS to be tested in the simulated biological fluid (aISF + BSA) to study their electrochemical stability. The stability test consists of a stress test (10,000 cycles of cyclic voltammetry [CV] in aISF + BSA) and exposure test (immersion in aISF + BSA for 2 weeks at 35°C). The electrochemical stability is observed by electrochemical impedance spectroscopy (EIS) during and after each test. Furthermore, to anticipate PEDOT stability in skin sensing applications, human skin lysate is added to the aISF to imitate on-skin sensing conditions. (Table 1)

2 RESULTS AND DISCUSSION

As electrochemical systems have been commonly adopted in many wearable sensor/on-skin sensing, it is important to observe the conducting polymer’s electroactivity in the simulated biological fluid, that is, aISF with BSA. Aside from simply observing the electroactivity, to serve the purpose of integrating conducting polymers in on-skin applications, it is crucial to also have good electrochemical stability in the aISF +1% wt/vol BSA. To demonstrate the proof of concept for these materials to work in a skin-like environment, human skin lysate will be added (1% wt/vol) to the aISF in the concluding electrochemical study.
PEDOTs electroactivity in simulated biological fluid

Three different variants of PEDOT coatings on ITO glass, each with different countions (1) PEDOT:TOS, (2) PEDOT:PSS, and (3) PEDOT:TOS/PSS were prepared to study their electroactivity. The electrolyte of aISF with addition of 1% BSA is used to simulate biologically relevant fluid. Since the thickness of the polymer coating will impact on the electrochemical performance, it is important to have similar thickness between samples. For all samples the thicknesses were 109 ± 2 nm. The electrical conductivity showed a large difference, from 2221 ± 48 to 2399 ± 154 to 216 ± 16 S cm⁻¹ for PEDOT:TOS to PEDOT:TOS/PSS to PEDOT:PSS respectively. X-ray photoelectron spectroscopy (XPS) analysis of the S 2p fine scans (Supplementary Figure S1) shows that the differences in the doping of PEDOT by TOS and/or PSS. The PEDOT:PSS shows the typical dominance of the PSS, which has been previously reported as a surface-rich layer on chemically prepared PEDOT.³⁰ Conversely, PEDOT:TOS shows the typical ratio of component peaks resulting in a doping level of approximately 24%. This is in good agreement with previously reported sulfonate to thiophene ratios reported for electropolymerized PEDOT:TOS of 0.22 (22%).³⁰ The co-doped polymer of PEDOT:TOS/PSS has a more involved fine scan, with peaks attributed to PEDOT, PSS, and TOS; at a total sulfonate to thiophene ratio of 0.47 (47%). The TOS to PEDOT ratio decreased to 15% (compared to the 24% for PEDOT:TOS), with PSS accounting for the remaining 32% of the sulfonate. This highlights the incorporation of PSS into the PEDOT:TOS, while maintain the electrical conductivity (that is an order of magnitude greater than the PEDOT:PSS variant).

In order to evaluate electroactivity of different PEDOT-based samples in the simulated biological fluid, two techniques in electrochemical analysis were adopted; CV and EIS. It is important to note that PEDOT behavior and performance in the aISF + BSA has not been previously reported. Hence in the first study (Figure 1(a)), electroactivity of PEDOT in the simulated biological fluid (dotted line) was compared with standard commercial phosphate buffered solution (PBS) (solid line). For all samples, the background currents are similar in both electrolytes, showing no hindrance of elements in the simulated biological fluid toward the electroactivity of all the PEDOT variants, regardless of the doping counterions used. In our work, neither the BSA present in the electrolyte, nor the variety

| Sample          | Components         | Line shape | FWHM  | Binding energy (eV) |
|-----------------|--------------------|------------|-------|--------------------|
| PEDOT:TOS       | PEDOT (S 2p₁/₂)    | SGL (30) T(1) | 0.77  | 163.5              |
| PEDOT (S 2p₃/₂) | SGL (30) T(1)     | 0.70       | 164.7 |
| Tos (S 2p₁/₂)   | GL (30)           | 1.2        | 167.0 |
| Tos (S 2p₃/₂)   | GL (30)           | 1.2        | 168.2 |
| H:Tos (S 2p₁/₂) | GL (30)           | 1.32       | 168.5 |
| H:Tos (S 2p₃/₂) | GL (30)           | 1.32       | 169.5 |
| PEDOT:PSS       | PEDOT (S 2p₁/₂)    | SGL (30) T(1) | 0.80  | 163.7              |
| PEDOT (S 2p₃/₂) | SGL (30) T(1)     | 0.76       | 164.9 |
| PSS (S 2p₁/₂)   | GL (30)           | 1.25       | 167.7 |
| PSS (S 2p₃/₂)   | GL (30)           | 1.25       | 168.9 |
| H:PSS (S 2p₁/₂) | GL (30)           | 1.35       | 167.2 |
| H:PSS (S 2p₃/₂) | GL (30)           | 1.35       | 168.4 |
| PEDOT:TOS-PSS   | PEDOT (S 2p₁/₂)    | SGL (30) T(1) | 0.80  | 163.5              |
| PEDOT (S 2p₃/₂) | SGL (30) T(1)     | 0.73       | 164.7 |
| Tos (S 2p₁/₂)   | GL (30)           | 1.2        | 167.0 |
| Tos (S 2p₃/₂)   | GL (30)           | 1.2        | 168.2 |
| PSS (S 2p₁/₂)   | GL (30)           | 1.35       | 167.7 |
| PSS (S 2p₃/₂)   | GL (30)           | 1.35       | 168.9 |
| H:S (S 2p₁/₂)   | GL (30)           | 1.30       | 168.5 |
| H:S (S 2p₃/₂)   | GL (30)           | 1.30       | 169.5 |

Abbreviations: PEDOT, poly3,4-ethylenedioxythiophene; PSS, polystyrenesulfonate; TOS, tosylate.
of constituents in the aISF imposed a barrier to PEDOT performance. This fits well with previous studies suggesting PEDOT can function well in bodily fluids.\textsuperscript{10,16,31} On the assumption of constant mass of PEDOT used (thickness is constant and test area is fixed), constant scan rate (mV/s), and constant voltage range in the CV, the specific capacitance ($C_p$) of the PEDOT is proportional to the area within the CV curves.\textsuperscript{32} This indicates that the PEDOT:TOS/PSS and PEDOT:TOS have the largest $C_p$ (ca. 30 F/g), with both being larger than the PEDOT:PSS (22.5 F/g).

The ability of PEDOT to transfer electrons in the aISF + BSA was observed by adding 0.5 mM ferri/ferrocyanide redox couple (Figure 1(b)). The cyclic voltammograms (at 10 mV/s scan rate) were recorded to give insight on electron transfer characteristic which may be affected by the different PEDOT variants and/or the constituents of the aISF + BSA. In general, all PEDOT variants showed typical CV profiles, implying the reversible process of electron transfer to/from the redox couple.\textsuperscript{33} However, notice that the maximum anodic current and peak potential are slightly different between the PEDOT variants. Figure 1(c) shows the trend of peak anodic current at various scan rates (10, 20, 30, 50, 70, and 100 mV/s). The trend of peak current versus the square root of the scan rate is commonly adopted to further qualify electron-transfer properties of the electrode-redox couple.\textsuperscript{34,35} Referring to the Randles-Sevcik equation (Supplementary Equation S1), peak currents will increase linearly as a function of the square root of the scan rate for reversible electron transfer. Figure 1(c) shows a linear function for all PEDOT variants with $R^2 \approx 0.996$–0.999. The same analysis applied to the peak cathodic current (not shown) for all PEDOT variants shows the same linear function with $R^2 \approx 0.987$–1.000. Linear regression with $R^2 \approx 0.99$ between peak current and the square root of the scan rate was achieved in other work using a working electrode of PEDOT:PSS-modified platinum in PBS containing 50 mM K$_3$Fe(CN)$_6$ solution.\textsuperscript{35} Their PEDOT:PSS was electropolymerized on screen printed platinum and achieved performance comparable to the bare platinum electrode in the PBS. Meanwhile, in our work, PEDOT:TOS, PEDOT:PSS, and PEDOT:TOS/PSS samples have showed similar linear relations, while immersed in the simulated biological fluid. Using Supplementary Equation S2, the effective surface area for the working electrodes are 0.69 cm$^2$ for PEDOT:TOS and PEDOT:TOS/PSS and 0.62 cm$^2$ for PEDOT:PSS. The variation of peak potential is influenced by the energy required for electron diffusion from the bulk solution toward the surface of the working electrode.\textsuperscript{33} Supplementary Figure S2 shows the difference of anodic peak potential and cathodic peak potential (peak-to-peak separation, $\Delta E_p$) over increasing scan rates. Here, changes of $\Delta E_p$ for PEDOT:PSS was larger compared to PEDOT:TOS and PEDOT:TOS/PSS. This may be related to the different size of the dopants (molecular vs. polymeric) causing a measurable shift in the redox potential.\textsuperscript{33} The similar behavior of PEDOT:TOS/PSS with PEDOT:TOS however suggests that TOS dominates the electrochemical behavior of both these polymer.

From the initial study, we have shown that all the PEDOT variants have good electroactivity and electron transfer ability in the simulated biological fluid. As mentioned earlier, it is important to observe PEDOT (electro-) stability in the biological fluid as preparation for on-skin application, which will be the focus in the next section.

2.2 | Electrochemical stability in aISF + BSA

Doped PEDOT has been shown above to in general have good electroactivity and electron transfer ability in the simulated biological fluid. The next critical step is to determine the polymer’s stability as a working electrode in the simulated biological fluid. To test the stability of PEDOT, two aspects were included, which are a stress test and an exposure test. The former will determine any impedance changes over continuous cycles while the
latter will determine changes upon exposure to the simulated biological fluid for extended periods at body relevant temperatures. The impedance value is derived from the EIS technique.

2.2.1 | Electrochemical stress test

An electrochemical stress test is conducted with the purpose to observe sustainability of the electrode’s performance over large numbers of continuous CV switching/cycles. In the stress test, 8000 cycles of CV with potential range from \(-0.6 \text{ V}\) to \(0.6 \text{ V}\) and scan rate of 200 mV/s were run continuously on all PEDOT working electrodes. The potential range is chosen to prevent further oxidation/reduction of PEDOTs with other ions in the electrolyte. The impedance values at 0, 500, 2000, 7000, and 8000 cycles were recorded to observe impedance change before, during, and after testing. The Nyquist plot constructed by real/imaginary impedance value on the x-axis/y-axis are presented in Figure 2(a). As compared to the small changes during cycling for PEDOT:TOS and PEDOT:TOS/PSS, note the impedance of PEDOT:PSS changes significantly, with evident expansion of the semicircle region (at the higher frequency range). The growth of this semicircle was also observed for PEDOT:TOS/PSS (Supplementary Figure S3a) but to a much lesser extent. The Nyquist plot can be modeled using a modified version of the Randles equivalent electrical circuit\(^{36}\) (with a constant phase element instead of the Warburg component) as shown in Figure 2(b), where the diameter of the semicircle is related to the charge transfer resistance between the electrolyte and the electrode, \(R_{\text{CT}}\). The other components of the equivalent circuit model (for PEDOT: PSS and PEDOT:TOS/PSS) are presented in Supplementary Figure S3b. The values of \(R_{\text{CT}}\) are compared in Figure 2(c) for the three PEDOT variants. Though it has been reported that PEDOT has remarkable stability across large numbers of electrochemical cycles,\(^{37}\) in this work \(R_{\text{CT}}\) increases in all samples during the stress test. In other words, the resistance for electron transfers to overcome in the system increases after repeated cycles. This is undesirable, as large \(R_{\text{CT}}\) inhibits the use of the material for repeated sensing applications. In the study herein, PEDOT:TOS and PEDOT:TOS/PSS present as better candidates for sensing in on-skin applications. The other components extracted from the equivalent electrical circuit model have rather constant values over the cycles (Supplementary Figure S3c).

Referring to Supplementary Figure S3a, it is interesting to note that between 7000 and 8000 cycles there is a shift to lower real impedance for both PEDOT:TOS and PEDOT:TOS/PSS, opposite to the increasing trend for the preceding 7000 cycles. Importantly, a 12 h pause in testing was added between 7000 and 8000 cycles. We suggest that during this time, the PEDOT begins to relax back toward its untested state (physical, chemical, electrochemical, etc.). To further observe this hypothesis, we introduce a further relaxation period of 3 h prior to conducting a further 2000 cycles. Supplementary Figure S4 shows the impedance plot and \(R_{\text{CT}}\) value at 7000, 8000 cycles and after the additional 2000 cycles (referred as reading at 10,000 cycles). It is observed that the impedance value reduces for PEDOT:TOS and PEDOT:TOS/PSS after the extra relaxation period while the impedance for PEDOT:PSS continues to increase. We suggest from this that PEDOT:TOS and PEDOT:TOS/PSS to a certain extent begin to recover their original electroactivity upon sufficient time of no electrical testing. While PEDOT:PSS

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**FIGURE 2** Impedance response of PEDOT during stress testing. (a) Measured impedance (in the form of a Nyquist plot) of PEDOT:TOS (black lines), PEDOT:PSS (blue lines), and PEDOT:TOS/PSS (orange lines) in biological media at 0 (solid), 500 (dotted), 2000 (dashed), 7000 (dashed-dot), and 8000 (dashed-dot-dot) cycles (aISF + BSA without any added ferri/ferrocyanide). (b) The Nyquist plot of PEDOT:TOS for experimental plot (solid) and modeled plot (round markers) using the modified Randles equivalent electrical circuit model. Inset shows circuit model consists of solution resistance, \(R_S\), charge transfer resistance, \(R_{\text{CT}}\), constant phase element, CPE, and double layer capacitance, \(C_{\text{DL}}\). (c) The \(R_{\text{CT}}\) extracted from the equivalent circuit model for the PEDOT variants. aISF, artificial interstitial fluid; BSA, bovine serum albumin; PBS, phosphate buffered solution; PEDOT, poly3,4-ethylenedioxythiophene; PSS, polystyrenesulfonate; TOS, tosylate [Color figure can be viewed at wileyonlinelibrary.com]
continues to change over subsequent cycles irrespective of the rest period (and undergoes continued changes in itself). Taking this into consideration, the next stability test will include a longer time interval between each impedance reading, hence may further testify to the recovering impedance property of PEDOT:TOS, PEDOT: TOS/PSS, and non-recovering property of PEDOT:PSS. In the exposure test, all PEDOT samples are immersed in aISF + BSA electrolyte in an incubator over 2 weeks at 35°C. As compared to the stress test where the CV cycles ran continuously, during the exposure period the impedance reading are measured periodically every two days. Since each reading has an interval of two days, this may give enough time for PEDOT to recover (or not) the impedance value before the next reading.

2.2.2 | Exposure test

In the exposure test, samples were immersed in the simulated biological fluid for a 2-week period in an incubator at 35°C; and the impedance measured every two days (Figure 3(a)).

For PEDOT:TOS and PEDOT:TOS/PSS there is little change in the Nyquist plot (in particular for PEDOT: TOS/PSS). Green et al. demonstrated electropolymerized PEDOT:TOS and PEDOT:ClO₄ were able to retain their electrochemical performance (measured as charge storage capacity) after immersion in 15% fetal bovine serum in cell growth media over a 2 week period. As mentioned earlier, the R_CT values for PEDOT:PSS slightly increase as compared to the other PEDOT variants. This is in agreement with the previous result in the stress test where the impedance of PEDOT:PSS increases over cycling/reading time, showing accumulated resistance of electron transfer. Similar to the previous section, the Nyquist plot is modeled using the modified Randles model and the R_CT presented in Figure 3(c). As shown in Supplementary Figure S5, applying the equivalent electrical circuit model to this data shows little change occurs in the other parameters (C_DL and CPE).

From these electrochemical stability tests, it can be understood that all the PEDOT variants can withstand (without failure) a large number of cycles and periods of exposure in aISF + BSA at body temperature. However, in the case of PEDOT:PSS, the impedance and R_CT increases with increasing cycles and exposure to aISF + BSA which may impacted its performance in skin-related applications such as wearable sensors. It is important to observe the material performance in near real conditions for skin by preparing the aISF with addition of skin content, which will be described in the next section.

3 | ELECTROCHEMICAL STABILITY IN THE PRESENCE OF HUMAN SKIN LYSATE

Previous sections have shown PEDOT electroactivity and stability in simulated biological fluid, consisting of aISF +1% wt/vol BSA. In order to prepare for on-skin application, 1% wt/vol human skin lysate was added to the aISF (no BSA) to more closely imitate the conditions within skin. The commercially available wearable devices are predominantly continuous glucose monitoring systems (GSM). Following the clinical protocol for the GSMs, the sensors need to be stable over a 24 h period. Hence, the possibility of using PEDOT as a wearable on-skin sensor requires the stability of PEDOT in aISF + skin lysate to be stably monitored over 24 h.

Figure 4(a) shows the Nyquist plot for PEDOT at 0, 2, 4, 6, 9, 12, and 24 h. Throughout this period, PEDOT films were immersed in aISF + skin lysate and placed on an orbital shaker, to incorporate the flow of liquid across the PEDOT as would be present in the in human body.
Similar to previous stability tests, the impedance for PEDOT:TOS and PEDOT:TOS/PSS remained relatively constant. The observed variation is attributed to experimental variations such as counter electrode positioning within the three-electrode electrochemical cell. Meanwhile, the semicircle region for PEDOT:PSS was still changing over time, as expanded upon in Supplementary Figure S6. This is aligned with the observation in the previous section, as well as the increase of RCT for PEDOT:PSS shown in Figure 4(b).

In summary, electroactivity and electrochemical stability of three PEDOT variants were studied in simulated biological fluids (aISF with either 1% wt/vol BSA or 1% wt/vol human skin lysate). All the PEDOT variants have similar electroactivity in aISF + BSA when compared to their electroactivity in standard PBS buffer. Good electron transfer ability for these PEDOT variants was determined from the linear relationship between anodic peak current and the square root of the scan rate when ferri/ferrocyanide was added to the aISF + BSA. By conducting a stress test and an exposure test, PEDOT was shown to be relatively stable when electrochemically cycling in the simulated biological fluid. More so, PEDOT:TOS and PEDOT:TOS/PSS showed quite constant impedance, between 0.1 Hz and 10 kHz, across the stress and exposure test—whereas PEDOT:PSS was observed to continually increase in (real) impedance with cycling. Modeling of the Nyquist plots showed an increase of the charge transfer resistance for PEDOT:PSS while the others were constant. The introduction of a pause in electrochemical cycling, to allow the system to relax, proved advantageous for the PEDOT variants that employed TOS. The pause in cycling indicated any changes in the impedance were reversible for PEDOT:TOS and PEDOT:TOS/PSS (but not PEDOT:PSS). Finally, the PEDOT variants were tested in aISF with added (1% wt/vol) human skin lysate. Again, PEDOT:TOS and PEDOT:TOS/PSS showed excellent electrochemical stability throughout the test. This suggests that PEDOT containing TOS may be most suitable for integration into wearable on-skin devices for electrochemical sensing or stimulation.

**FIGURE 4** Electrochemical performance of PEDOT in aISF with skin lysate. (a) Nyquist plot of the three PEDOT variants in aISF with 1% wt/vol skin lysate periodically measured over a 24 h period. (b) A zoomed Nyquist plot for PEDOT:TOS and PEDOT:TOS/PSS (black), and PEDOT:TOS/PSS (orange). aISF, artificial interstitial fluid; PEDOT, poly3,4-ethylenedioxythiophene; PSS, polystyrenesulfonate; TOS, tosylate [Color figure can be viewed at wileyonlinelibrary.com]
5 | MATERIALS AND METHODS

ITO coated glass (<15 Ω/square; Kaivo Electronics) was used as substrate for all PEDOT samples and variants. The substrate was sonicated in water and ethanol solution for 15 min to remove any foreign materials prior to any fabrication process. Next, substrates were air plasma treated (PDC-32G, Harrick Inc.) for 5 min for VPP process (PEDOT:TOS and PEDOT:TOS/PSS) and 20 min for spin-coating (PEDOT:PSS).

5.1 | Vapor phase polymerization PEDOT:TOS

The fabrication of the PEDOT:TOS by vapor phase polymerization (VPP) was the same as previously published work. First, oxidant solution was prepared containing: 257 mM Fe(III) TOS (H. C. Starck as a 54 wt% solution in butanol -Baytron CB 40) and 58 mM triblock copolymer poly(ethylene glycol—propylene glycol—ethylene glycol) (PEG-PPG-PEG, Mw = 5800 g mol⁻¹, Sigma-Aldrich) in a 2:6:1 vol/vol mixture of ethanol to butanol as the solvent carrier. The oxidant solution was spin-coated (400B-6NPP, Laurell Technologies Inc.) on the ITO coated glass substrate for 25 s at 1500 rpm. To remove excess solvent, samples were placed on hotplate for 30 s at 70°C before moving into the polymerization chamber (Binder Vacuum Oven-VD 115) for 1 h at 23°C and 45 mbar. The EDOT monomer is heated to 35°C. Finally, samples were rinsed using ethanol and dried with low-flow air gun to remove excess materials.

5.2 | Spin-coated and methanol treated PEDOT:PSS

The fabrication of the PEDOT:PSS followed the protocol of previous work in ref. 44. Poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) (PEDOT:PSS, 1.3 wt% dispersion in H₂O, conductive grade, Sigma Aldrich) was filtered through a 0.45 mm syringe filter to remove large aggregates. The solution then spin coated on air plasma treated ITO-glass substrate for 25 s at 800 rpm before annealing on a hot plate at 120°C for 30 s. The spin coat-anneal procedure was repeated three times in order to get film thickness comparable to PEDOT:TOS before a final anneal at 120°C for 30 min. To increase PEDOT:PSS’s conductivity and stability in water, the sample was treated in methanol by immersion for 15 min, then annealed on a hot plate at 140°C for 5 min to remove excess methanol.

5.3 | Hybrid PEDOT:TOS/PSS by electrochemical redox

Previously prepared VPP PEDOT:TOS was used to prepare hybrid (co-doped) PEDOT. First, PEDOT:TOS was electrochemically reduced in 0.1 mM Sodium p-toluenesulfonate (NaTOS, Sigma Aldrich) solution using 10 CVs from −1 V to 0 V to −1 V with time interval of 10 s between voltage change and hence 20 s for 1 cycle. Keeping potential range at negative potential will allow only reduction of PEDOT to happen during the CV cycles, thus removing the TOS ions into the electrolyte. Then the reduced sample was quickly rinsed with deionized water (DI) to remove surface contamination of NaTos. The sample then electrochemically oxidized in 0.1 mM poly(sodium styrenesulfonate) (NaPSS, Mw = 77,000 g mol⁻¹, Sigma-Aldrich) solution using 10 CVs from +1 V to 0 V to +1 V with time interval of 10 s between voltage change and hence 20 s for 1 cycle. For oxidation of PEDOT, the potential range is kept at positive potential only, hence allowing PSS ions to electrostatically interact with the oxidized PEDOT. Again, the sample was cleaned using DI water. Color changes from dark blue to light blue was observed during the oxidation of PEDOT:TOS with PSS as a visual cue to the uptake of PSS.

5.4 | Simulated biological fluid preparation

The aISF was prepared as referred to ref. 45 containing (mM) 1.53 CaCl₂, 5.55 glucose, 10 Hepes, 0.69 MgSO₄, 1.67 NaH₂PO₄, 3.48 KCl, 107.7 NaCl, 9.64 NaC₆H₁₁O₇ and 7.6 sucrose at pH 7.4. Preparation of the simulated biological fluid was using aISF loaded with 1% wt/vol bovine albumin serum. All chemicals were purchased from Sigma Aldrich.

5.5 | Skin lysate preparation

Excised human skin from abdominoplasty was sourced from Calvary North Hospital, North Adelaide, Australia and approval was obtained University of South Australia, Research Ethic and Integrity (Protocol No: 2000745). The skin was cleaned with saline and fat was trimmed prior to the digestion. The clean excise skin was cut into pieces (35 mg each) and placed into a tube with the 3.2 mm stainless steel beads. For each sample, 70 μl of saline was added into the tube. The tube then closed tightly before being placed in a Bullet Blender (Lite bt12lt, Next Advance) and run at speed setting 12 for 5 min.
After the run, the tubes were centrifuged at 13000 rpm for 2 min and the supernatant was transferred to an Eppendorf tube. 0.5 mg total of human skin was added to 50 ml of aISF solution.

5.6 | Thickness measurements

Thickness (t) of the samples were measured by a Bruker Dektak XT profilometer across a scratch in the polymer layer (scan length of 1000 μm and scan duration of 20 s, using the piezo setting for a 6.5 μm height). The scan was performed using a stylus force equivalent to the mass of 3 mg and the stylus of 12.5 μm in radius. A lateral scan resolution of 0.66 μm/point was used for all the measurements.

5.7 | Electrical conductivity measurements

A four-point probe from Jandel instruments, U.K. were used to measure the sheet resistance of the PEDOT samples, where these films are having uniform thickness. A van der Pauw measurement was carried out to calculate the sheet resistance (Rₑ) of the samples by applying a constant current of 3 mA and recording the potential between the electrodes. By using (σ = 1/ Rₑ * t), the electrical conductivity (σ) of the films were calculated.

5.8 | X-ray photoelectron spectroscopy

All spectra were acquired using a Kratos Axis Ultra DLD spectrometer. The x-ray source used was an Al monochromatic Kα source with energy 1486.6 eV. Power output of the source was 225 W obtained by using source parameters 15 kV and 15 mA. The analysis chamber vacuum during spectral acquisition was 4x10⁻⁸ mbar. The charge neutralizer (Kratos charge neutralizer) was turned on and operating with neutralizer parameters: filament current 2.0 A, charge balance 3.6 V and filament bias 3.5 V. Instrument calibration used metallic gold and copper. The work function was set to −1.441 V giving an Au 4f7/2 binding energy position of 84.03 eV. The spectrometer dispersion was set so that Cu 2p3/2 was located at the binding energy of 932.69 eV. Instrument calibration procedure was according to AS ISO 1547:2006-Surface chemical analysis-X-ray photoelectron spectrometers-calibration of spectrometer energy scale. The resulting metallic Ag 3d₃/₂ was found to be located at 368.26 eV. All spectra were collected over a rectangular analysis area with dimensions 300 × 700 μm. Wide scan spectra were collected using the pass energy 160 eV. Narrow scan spectra were collected with a pass energy of 20 eV. Spectra were charge corrected to adventitious carbon located at 284.8 eV. All spectra were analyzed using CasaXPS version 2.3.18PR1.0 (Casa Software Ltd, Wilmslow, Cheshire, UK).

The spectral shape of the S 2p was aligned with C—C (hydrocarbon) peak at 284.8 eV in the associated C 1 s fine scan (not presented). In the S 2p spectra, two doublets were used for fitting with the hybridization of the 2p orbital as 1.2 eV and the area of S 2p₁/₂ being half that of S 2p₃/₂ respectively. The asymmetric line shape is used for fitting the S 2p spectra of PEDOT chain defining the metallic behavior of the delocalized positive charges across the polymer chains as was suggested by Zotti et al. The specific fitting parameters for S 2p are as follows;

5.9 | Device characterization

The electrochemical analysis is performed using an IVIUM potentiostat (IVIUM-n-Stat, IVIUM Technology) with a standard three electrode configuration consists of Ag/AgCl (3 M NaCl) as a reference electrode, platinum wire as counter electrode and PEDOT/ITO as working electrodes. The investigation of PEDOT electroactivity in simulated biological fluid was performed by running CV from −0.2 V to 0.6 V. The stress testing was performed over 10,000 CVs in the simulated biological fluid from −0.6 V to 0.6 V relative to the reference electrode at constant rate of 200 mV/s and 5 mV potential step. The exposure test was performed over a 2-week period where PEDOT variants were immersed in the simulated biological fluid in an incubator set to a constant temperature of 35°C. IVIUM potentiostat was used to perform EIS for testing with a 10 mV root-mean-square (RMS) sinusoidal signal, 0.2 DC bias and frequency swept from 0.1 Hz to 10 kHz.

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