Wien Oscillator Using Organic Enzyme-Chemiresistors for Fused Measurement of Glucose and Lactate

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1. Introduction

Multi-sensor data fusion is the process of integrating data from multiple sensors (or biosensors) into a single useful actionable, analytical (or bioanalytical) output. The motivation for multi-sensor data fusion is simple: through synergy, the efficacy of fused data for decision making is likely to be higher than that provided by individual components that make up the fused data. Data fusion techniques and their implementation are commonly found across multiple industries and technologies, including image processing, global positioning system (GPS) tracking, and vehicle traffic mapping. As the technology reaches maturity, data fusion is now being developed and applied to multi-parametric sensor systems, such as wearable wireless body sensor networks (WSBNs) or implantable biosensor systems. One such implantable biosensor system has been developed for the management of hemorrhaging trauma through measurement of key biomarkers. Due to systemic complications arising from hemorrhage following trauma, among them insulin resistance, an increase in lactate (hyperlactatemia) associated with tissue hypoxia, and a subsequent decrease in pH (acidosis), a systemic approach must be adopted to properly assess, triage, and resuscitate the hemorrhaging patient. One approach being pursued is the development of rapidly deployable, minimally invasive biosensors to monitor a suite of physiologically relevant biomarkers for the stratification of patients and for their guided resuscitation. The demands of austere environments, such as total body sensor networks for physiological (diagnostic biosensors) and biomechanical (force and motion) systems require data fusion through several algorithms. A novel, device-level method of sensor data fusion is presented. This biomimetic concept is demonstrated through the development of glucose and lactate sensitive chemiresistors in a biologically responsive Wien bridge oscillator circuit. Biocompatible and bioresponsive polymers suitable for integration of molecular recognition and transduction yield stimuli-responsive chemiresistors. The analyte-responsive chemiresistors are electroconductive hydrogels that separately incorporate the enzymes glucose oxidase and/or lactate oxidase that are conjugated with single-walled carbon nanotubes (SWCNTs) and embedded in a percolating network of conductive polymer (polypyrrole). The input of the system is the chemical potential of the analytes, glucose and lactate, acting through the biological activity of immobilized enzymes, and the output of the system is a single sinusoidal wave from which the concentrations of glucose and lactate can be deconvoluted from the amplitude and DC offset, respectively. This engineered system, based on soft bioelectronic circuit elements, aims to offer simultaneous, real-time analyte biosensing at a biomolecular level in a move toward biologically responsive circuits and eventually, bioanalytical systems suitable for indwelling in human tissue for extended periods of time.
deep space, the battlefield, mass triage following major manmade or natural disasters, and remote or rural areas, necessitate the development of fused actionable data over the use of discrete vital signs. A typical data fusion process occurs, as shown in Figure 1A, where information from individual sensors are fed into a data fusion algorithm, which outputs a single fused value of some significance to make a decision or to score the severity of a traumatic injury. An example is the injury severity score (ISS), which aggregates the assessed injury to six body regions and correlates with mortality and morbidity. In this scheme, sensor data fusion is achieved at the software level, where several hardware transmission lines of data are fed into a microprocessor to be analyzed and fused. Data fusion algorithms, such as artificial neural networks (ANNs) or principal component analysis (PCA), are commonly used as software-side decision making algorithms for array biosensors, such as electronic noses or electronic tongues.

To demonstrate the use of sensor-level data fusion, a Wien bridge oscillator circuit with appended direct current (DC) offset was designed, fabricated, and validated (Figure 2A). A Wien bridge oscillator is a simple circuit that generates a constant sine wave without any sourced input using both positive and negative feedbacks. In this work, two resistors in the system were replaced with biologically responsive chemiresistors based on electroconductive hydrogels; one is hydrogel whose impedance varied with the concentration of glucose, and one whose impedance varied with the concentration of lactate.

The magnitude of the two resistors is capable of controlling two aspects of the output signal: 1) peak-to-peak alternating current (AC) signal magnitude ($V_{AC}$) and 2) DC offset bias voltage ($V_{DC}$). As the impedance of either resistors changed (within an engineered window), an independent change of the output signal was possible, either as a change in $V_{AC}$ ($\Delta$ [glucose]) or $V_{DC}$ ($\Delta$ [lactate]) (Figure 2B).

To date, there are only a modest number of reports on simultaneous dual- or multi-sensing of analytes through the use of data fusion techniques at the molecular level—the advantages of which are obvious: a single device enabled for continuous measurement of multiple key physiological analytes (e.g., glucose, lactate, pH, potassium, and pO$_2$) could have a major impact on the understanding and management of the hemorrhaging trauma patient. Some specific and interesting recent examples include: Liao et al. developed a dual-mode glucose/pH biosensor that uses a pH-responsive tin oxide (SnO$_2$) and glucose oxidase field-effect transistor. Liu et al. developed a simultaneous dual-mode pH and O$_2$ sensor utilizing both amperometric and potentiometric detection. Bui et al. reported the simultaneous dual detection of nitrate and mercury in water via disposable potentiometric biosensors.

In this body of work, a bioelectronic Wien bridge oscillator system was engineered to incorporate two analyte sensitive chemiresistors fabricated using conductive polymer polypyrrole (PPy) and supramolecular bioactive conjugates of enzymes glucose oxidase (GOx) or lactate oxidase (LOx) and single-walled carbon nanotubes (SWCNTs) in photo-crosslinkable hydrogels. After the bioelectronic system was modeled in Multisim/LabVIEW and tested in a benchtop setting, the feasibility of utilizing the fused data signal rather than individual feature values to classify a patient’s trauma severity was studied by training and testing an ANN in MATLAB.

![Figure 1. A) Data fusion at the software level: data on separate transmission lines from various sensors serve as inputs to a software system, where each input is fused using some algorithm (ANN, PCA, Kalman filter, etc.) and used to arrive at some actionable output. B) Data fusion at the device level: data from various sensors are fused at the instrumentation level and broadcast as a single signal, which can either be defused into its individual, discrete components or used as a single data set to arrive at some actionable output.](image-url)
2. Results and Discussion

2.1. In-Silico Modeling of Bioelectronic Wien Bridge Oscillator

From in-silico circuit modeling using Multisim and LabVIEW, Figure 3A,B shows the residuals of predicted values of $R_{\text{glu}}$ and $R_{\text{lact}}$ calculated from measuring the peak-to-peak voltage ($V_{\text{AC}}$) and DC offset ($V_{\text{DC}}$) of the simulated output wave and back-calculating the input resistance values. Using the paired t-test, both $R_{\text{glu}}$ and $R_{\text{lact}}$ values were not statistically different from the actual values over the ranges measured ($P < 0.001$), indicating that the equations used to back-calculate resistance values were valid. Furthermore, the $R^2$ values calculated from linear regression of the glucose chemiresistor were 0.9913 ($R_{\text{glu}}$ only) and 0.9933 ($R_{\text{real}}$) and the $R^2$ values calculated from linear regression of the lactate chemiresistor were 0.9839 ($R_{\text{lact}}$ only) and 0.9890 ($R_{\text{real}}$), indicating fidelity of the modeled response of the circuit behavior. Incorporation of the Randles model ($R_{\text{real}}$) resulted in a modest increase in $R^2$; however, it did not address the patterned residual shown in Figure 3A, indicating that a higher order model was required for appropriately modeling very high and very low concentrations of glucose. The nonlinear response of the $R_{\text{glu}}$ value is likely due to the parasitic capacitance at the electrical double layer of the electrode/hydrogel interface and the hydrogel membrane itself.\(^{15}\) As Equation (3) (calculation of $R_{\text{glu}}$) does not consider the effect of current draw parasitic capacitance, a correction factor utilizing the known reactance of the $Q_{\text{DL}}$ can be used.

Concentrations of glucose and lactate calculated from predicted chemiresistor values plugged into the Hill equation are shown in Figure 3C,D. As shown in the Clarke error grids, the model can appropriately back-calculate the actual glucose and lactate concentrations. In the glucose error grid, 100% of points calculated using the $R_{\text{real}}$ values of the Randles circuit fall into Group A, whereas only 46% of the points fall within Group A when using only $R_{\text{glu}}$. Similarly, in the lactate error grid, 100% of points calculated using the $R_{\text{real}}$ value of the Randle circuit fall within Group A, whereas only 81% of the points fall within Group A when using only $R_{\text{lact}}$. For both models, using the paired t-test, both predicted glucose and lactate concentrations were not statistically different from the actual values ($P < 0.01$), indicating that the use of the Hill model to calculate concentrations of analyte from the calculated resistances was valid.

2.2. Electrochemical Characterization of Chemiresistor Hydrogels

The chemiresistor biosensors were first characterized by electrochemical impedance spectroscopy (EIS) before incorporation into the Wien bridge oscillator bioelectronics system. An equivalent circuit analysis was completed using the modified Randles circuit ($R_{\text{M}}(Q_{\text{DL}},R_{\text{CT}})$), where the first series resistance, $R_{\text{M}}$, is associated with the high-frequency membrane or solution resistance. The value of $Q_{\text{DL}}$ (constant phase element) models an imperfect capacitor and is associated with the double layer capacitance of the electrode–electrolyte interface and where $n$ represents the deviation from ideality ($0 \leq n \leq 1$). In parallel with the $Q_{\text{DL}}$ is $R_{\text{CT}}$, the resistance associated with the transfer of charge across the polymer/platinum electrode interface. Charge transfer resistances extracted from the impedance spectra of glucose and lactate chemiresistors of increasing complexity are shown in Figure 4A,B, respectively. The control hydrogels (no enzyme or inherently conductive inclusion) show no response to increasing concentrations of analyte, due to the lack of any enzyme and a means of transduction. Interestingly, the hydrogels loaded with only enzyme showed similar impedances to the control hydrogel but were sensitive to analyte at low frequencies, approaching DC, reflecting the contribution of amperometric discharge of $H_2O_2$ at the platinized platinum electrodes (Figure 5A). This response is likely due to the movement of charge of the product of the enzymatic reaction, $H_2O_2$, transferring charge at the electrode, catalyzed by the platinum nanoparticles.\(^{16}\) Incorporation of polypyrrole:polystyrene sulfonate (PPy:...
Figure 3. A,B) Residual plots of calculated glucose and lactate resistances and C,D) Clarke error grids of analyte concentrations calculated from the LabView circuit model using both the chemiresistor and the Randles cell model.

Figure 4. A,B) Charge transfer resistances extracted from impedance spectra of glucose and lactate chemiresistors measured in 0–50 mM of glucose or lactate ($n = 3$). C,D) Simultaneous and concurrent peak-to-peak amplitude changes due to glucose concentrations and DC voltage offset due to lactate concentrations ($n = 3$). E,F) Calibration curves of glucose and lactate chemiresistors ($n = 3$).
PSS) nanoparticles serves to introduce yet another pathway of charge transfer as the H$_2$O$_2$ produced by the GOx competitively serves to oxidize the polymer, thereby increasing its conductivity and the inherently conductive polymer then serves as a mediator (Figure 5B). Incorporation of carbon nanotubes (CNTs) results in decreased impedance at low frequencies due to the conductive nature of the CNTs. Incorporation of CNTs serves to decrease overall charge transfer resistance while retaining the ability of H$_2$O$_2$ to oxidize at the platinum electrode surface, or to facilitate direct electron transfer from the active site of the enzyme directly to the electrode (Figure 5C). Besteman et al. fabricated a similar system, in which overall conductance of CNTs conjugated to GOx decreased upon introduction of glucose. The values of chemiresistor impedances at 1.0 Hz (sampling frequency of Wien bridge oscillator) were used to dictate values of solid-state resistors in the Wien bridge oscillator circuit, calculated to sustain oscillations over a large change in chemiresistor impedances.

2.3. Chemiresistor Biosensor Deployment

The Wien bridge oscillator was implemented using the enzyme-CNT|PPy composite system. Simultaneous and concurrent peak-to-peak amplitude changes due to glucose concentrations and voltage offset changes due to lactate concentrations are shown in Figure 4C,D, respectively. Approximate changes in chemiresistor values were calculated in real time in LabVIEW using the measured AC/DC signal and Equation (3) and (4). As expected, increasing concentrations of glucose resulted in an increased peak-to-peak voltage at the output of the Wien bridge oscillator as dictated by the relationship of the resistors in the feedback loop (decreasing $R_{glu}$ increases output signal amplitude). The DC offset voltage measured decreased with increasing concentrations of lactate as dictated by the voltage divider system (decreasing $R_{lac}$ increases voltage drop over $R_7$). Calibration curves arising from the biotransducers were calculated and are shown in Figure 4E,F. The key bioanalytical parameters extracted from a fit of the Hill equation are shown in Table 1. Finally, the Clarke error grids were calculated, as shown in Figure 6A,B.

The chemiresistor sensitivities were calculated by linear regression of the data presented in the Clarke error grid, resulting in a calculated sensitivity of $b = 39.9$ mV$_{AC}$ mM$^{-1}$ for glucose ($R^2 = 0.9571$) and $b = 31.8$ mV$_{DC}$ mM$^{-1}$ for lactate ($R^2 = 0.9781$).

| Table 1. Hill equation parameters of glucose and lactate sensors deployed in bioelectronic Wien bridge oscillator system (Hill coefficient and $K_0$). Limit of detections was calculated using Equation (1). Response time was measured as the average time to steady-state response. Sensitivity was measured by linear regression of predicted data shown in Figure 6A,B. |
|----------------------------------|-----------------|-----------------|-----------------|
| GOx|CNT-PPy | LOx|CNT-PPy |                 |
| Hill coefficient [h]             | 2.17            | 2.55            |
| $K_0$ [mM]                       | 6.20            | 3.05            |
| Limit of detection [mM]          | 1.15            | 0.54            |
| Response time [s]                | 58.1            | 86.8            |
| Sensitivity [mV mM$^{-1}$]       | 39.9            | 31.8            |
The in vitro biocompatibility of the molecularly engineered poly-2-hydroxyethyl methacrylate (p(HEMA)) based hydrogels used in this work has been established in other published works through cell culture, protein adsorption, and surface analysis.\[26,27\]

2.4. ANN Analysis

After the Wien bridge oscillator bioelectronic sensor system was validated, an ANN was trained, validated, and tested on three types of input data: 1) processed data in the form of a two feature vector (concentration of glucose and lactate); 2) data corresponding to the extracted features of interest of the sinusoidal wave output from the Wien bridge oscillator in the form of a two feature vector (extracted values of $V_{AC}$ and $V_{DC}$); and 3) fused data in the form of an n-feature, time-dependent waveform of the sinusoidal output of the oscillator, which carried within it data corresponding to both concentrations of glucose and lactate in a single wave. Each set was trained to classify the input data as “safe,” “elevated,” or “surgical life saving intervention (LSI) or death,” depending on the extent or glycemia and lactatemia.

Before a comparison of validation errors was carried out between the three data types, the number of samples of the 1.0 Hz fused-data sine wave was studied. In particular, the number of samples (sampled at 10 Hz) of the wave was varied from 10 to 100 (1 period to 10 periods of the 1.0 Hz fused data) and used to train, validate, and test. The mean square errors (MSEs) of the validation data sets at 50 epochs are shown in Figure 7A. The highest MSE corresponded to the data feature size of 10 samples (1 period of a sine wave), which decreased and leveled out at 30–50 samples (3–5 periods). Increased samples (6–10 periods) resulted in increased MSE and a less accurate neural network. Therefore, for comparison of the fused data to the other data types, the 30-sample size feature vector (3 periods of 1.0 Hz data) was chosen.

Each system was trained, tested, and validated at 50 epochs as well as the final epoch (final epoch is variable between feature set size). The MSE of each system is compared in Figure 7B. Using the Tukey’s honest significant difference ($p < 0.05$) test to compare all means at 50 epochs, there existed no statistically

![Figure 6](www.advintellsyst.com) Clarke error grids of deployed A) glucose and B) lactate chemiresistors in the Wien bridge oscillator system.
significant different pairs between the data types, indicating that in comparison with traditional data formats, an unprocessed sinusoidal signal carrying convoluted data of two analytes was able to perform at comparable accuracies in the neural network when compared with the more traditional discrete data formats. At this time, implantable biosensor systems capable of continuously sensing analyte concentrations (specifically glucose) are commercialized and approved for use by the Food and Drug Administration. Whereas most of these sensors are designed for diabetics to monitor their glucose (Dexcom G6, Abbot Freestyle Libre, Senseonics Eversense), future development of implantable electrodes carrying multiple chemistries (multiple enzymes or biorecognition elements) is being realized. This work serves to underline a new method of gathering data from multiple biosensing interfaces and utilizing that data to alter electrical parameters of a continuous sinusoidal wave. Further advancements can lead to more parameters being made for use in a single wave (such as the frequency of the waveform), making a single waveform capable of carrying a multitude of information gathered at the molecular level. As these bioelectronics become sophisticated, the way that the data are used to classify patient status must be equally sophisticated. Here, we have shown a simple “safe,” “elevated,” or “surgical LSI or death” class to demonstrate the feasibility of such an approach. As the understanding of the body’s physiochemical response to a traumatic event is better understood through research, more sophisticated neural network algorithms can be designed, giving a physician a single score of the patient’s health.

3. Conclusion

A biologically responsive Wien bridge oscillator circuit was developed using a combination of traditional solid-state resistors and two enzyme chemiresistors, sensitive to glucose and lactate, respectively. Biologically responsive (glucose and lactate sensitive) electroconductive hydrogels were fashioned by incorporating CNTenzyme conjugates with conductive PPy:PSS incorporated into biocompatible hydrogels. The modeled system, which used a combination of electronic analysis of circuitry and commonly used enzymatic rate equations, was capable of accurately deconvoluting and depicting concentrations of the analytes over a wide range of concentrations. In comparison with more traditional data types (discrete data such as those fed from individual sensors into a microprocessor), the fused data performed comparably, as shown by comparison of MSEs in a trained ANN. The concept of using fused data (specifically fused at the biomolecular and device level) to create simple electronic signals capable of carrying information relating to multiple analytes marks a step toward design of novel bioelectronics capable of acting as a closed-loop system within a biological matrix. Future work incorporating more analytes of interest can be designed with even more complex measurable variables in the transmitted signal, such as sine wave frequency and magnetic field intensity.

4. Experimental Section

Materials: Hydrogels were formulated from mixtures of the base monomer 2-hydroxyethylmethacrylate (HEMA) (43.25 mol%), base monomer 2-hydroxypropylmethacrylamide (HPMA) (45.25 mol%), N-tris(hydroxymethyl)methyl acrylamide (HMMA) (5.0 mol%), poly(ethylene glycol) monomethacrylate (PEG(360)MA) (5.0 mol% repeat units), crosslinker tetra(ethylene glycol)diacrylate (TEGDA) (1.0 mol%), pre-polymer to control viscosity, poly(N-vinylpyrrolidone) (pNVP) (molecular weight = 1.3M) (2.0 mol% repeat units), and the photoinitiator, 2,2-dimethoxy-2-phenylacetophenone (DMPA) (0.5 mol %), all from Sigma-Aldrich (St. Louis, MO, USA). Hydrogels were rendered conductive by inclusion of 1.0 wt% PPy nanoparticles (Sigma-Aldrich), SWCNTs (purity, 95 wt%), glucose oxidase (EC 1.13.1.4 from Aspergillus niger, G7141-250KU, type X-S, 146,000 units g⁻¹ solid), and lactate oxidase (EC: 1.13.12.4 from Pediococcus sp., 20,000 units g⁻¹ solid) were purchased from Sigma-Aldrich and used to create supramolecular conjugates (SWCNT-GOx, SWCNT-LOx). Lithium-L-lactate and D(+)-glucose (Sigma-Aldrich) were used to create dose response solutions from 0.0 to 50.0 mM in 0.01 M phosphate-buffered saline (PBS). Ultracentrifuge tubes (OakRidge Bottle, polycarbonate 16 x 83 mm and polypolyethylene sealing caps) were purchased from Fisher Scientific (Pittsburg, PA, USA). Polyethylene glycol monomethacrylate (HMMA) (5.0 mol%) 1.3M solid were used were assigned a 1% tolerance. Upon characterization of the circuit itself, LabVIEW 2016 (National Instruments, Austin, TX) was used to
To more accurately model the in situ behavior of the polymer chemiresistors, the Randles circuit \( R(QR) \) was used to replace each individual chemiresistor \( R_{\text{glu}} \) and \( R_{\text{lac}} \) in the model (Figure 2A, inset). The Randles circuit is a more appropriate model as it is able to accurately depict individual processes occurring in the chemiresistor (membrane resistance, charge transfer resistance, and double layer capacitance). Analysis using the Randles circuit allows for deconvolution of the real \( (Z_{\text{re}}) \) component of the chemiresistor while accounting for parasitic capacitance inherent to all electrode interfaces.

**Bioelectronics Hardware and Circuitry:** The bioelectronics system, which comprised of an Arduino Uno R3 microcontroller, a single 9 V battery, HC-05 Bluetooth transmitter, a 3D printed housing, and several electronic components [op-amp, resistors, capacitors, switch, push button, and female micro-universal serial bus (USB) plug], was visualized in a SolidWorks render in Figure 8A,B. The wiring diagram of the Wien bridge oscillator circuit and accompanying components is shown in Figure 8B. 

Supporting Information. The circuit was built using surface mount components on a printed circuit board (PCB), which interfaces directly with the ports of the Arduino. The Bluetooth transmitter, switch, and female micro-USB plug were soldered directly to the PCB and placed on top of the Arduino. The Arduino/PCB was housed in a 3D printed polylactic acid (PLA) casing. The final dimensions were 32 x 58 x 75 mm. The final bioelectronics system is shown in Figure 8C.

**Chemiresistor Hydrogel Fabrication:** Supramolecular conjugates of SWCNT, GOx, and LOx were fabricated according to previously published procedures. In brief, aqueous suspensions of SWCNT (5 mg) and enzyme (10 mg) were prepared in 5.0 mL deionized water. Suspensions were created at 4 °C by ultrasonication using a QSonica Q500 Tip Sonicator (Newton, CT, USA) outfitted with a 1/16” microtip probe. Ultrasonication was carried out in a pulse mode (10 s on, 10 s off) at 30% amplitude for 5 min. Conjugates were collected from the supernatant and were ultracentrifuged (30 000 x g for 1 h) in an Eppendorf 5424 Centrifuge (Hamburg, Germany). Following centrifugation, samples were stored at 4 °C.

Four hydrogel cocktail samples were made for either enzyme: 1) a control gel (no enzyme or conductive inclusion); 2) a gel with only enzyme; 3) a gel with CNT-enzyme conjugates; and 4) a gel with CNT-enzyme conjugates with the conductive inclusion of PPy:PSS. PPy:PSS nanoparticles were incorporated at 1.0 wt%, and SWCNT-GOx or SWCNT-LOx were incorporated at 1.0 wt%.
supramolecular conjugates were incorporated at 1 mg mL\(^{-1}\). Hydrogel cocktails (100 µL) were pipetted between two platinum parallel plate electrodes (electrode spacing: 2 mm, electrode working area: 1.5 cm\(^2\)) that were first subjected to deposition of platinum nanoparticles at a charge density of 50 mC cm\(^{-2}\) through previously published procedures that produced platinumized platinum electrodes (Figure 2A, inset).[38] The hydrogels were exposed through the edge of the assembly for 5 min to UV within a CX-2000 UV Crosslinker (UVP, LLC, Upland, CA, USA) to initiate polymerization of the hydrogel constituents. Following crosslinking, hydrogels were hydrated in 0.01 M PBS for 4 h. The chemical structure schematic of the crosslinked hydrogel and the roles of each component is shown in Figure 9.

**Figure 9.** Schematic of hydrogel constituents used for chemiresistor fabrication.

Wien Bridge Oscillator Bioelectronics Deployment: Following hydration, fabricated chemiresistors were interrogated using EIS in solutions of increasing concentration of glucose/lactate (0–50.0 mM). Impedance spectra were recorded from 0.1 Hz to 1.0 MHz using a 10 mVPP sine wave using a VersaStat 4 (Princeton Applied Research, Oak Ridge, TN, USA). Before measurements, hydrogel electrodes were equilibrated for 30 min in each solution. Equivalent circuit analysis of acquired impedance spectra was completed using ZSimpWin (v3.60, Princeton Applied Research, Oak Ridge, TN, USA) with the Randles circuit (R(QR)) model.

Following characterization of the glucose and lactate chemiresistors, the electrodes were placed in the Wien bridge oscillator biosensor system and subjected to a dose response in the physiological ranges of each analyte: 0–10.0 mM glucose and 0–4.0 mM lactate. The sinusoidal wave output from the system was transmitted via Bluetooth from the bioelectronics system and received in LabVIEW 2016 (National Instruments, Austin, TX). The measured \(V_{AC}\) and \(V_{DC}\) were used to calculate the apparent resistance of the chemiresistors. The resistances were then used to calculate the concentration of each analyte using the Hill model (Equation (2)).[^31] Accuracy of the biosensor system was plotted as Clarke error grids.

**ANN to Classify Extent of Trauma:** A simple, two-layer feed forward ANN was coded in MATLAB R2019a to study the ability to classify three types of data: 1) data that have already been processed (Figure 1A) in the form of a two feature vector (concentration of glucose and lactate); 2) data corresponding to the extracted features of interest of the sinusoidal wave output from the Wien bridge oscillator in the form of a two feature vector (extracted values of \(V_{AC}\) and \(V_{DC}\)); and 3) nonprocessed, fused data in the form of \(n\)-feature waveform \( (n = \text{number of samples of sinusoidal wave at a sampling rate of } 10\text{ Hz}; 10–100\text{ samples were studied}) \) of the sinusoidal output of the Wien bridge oscillator, which carried with data corresponding to both concentrations of glucose and lactate in a single wave (Figure 1B). The neural network was coded with a hidden layer of 10 neurons utilizing a sigmoid activation function and an output layer utilizing a linear neural output corresponding to three classes of interest: 1) safe: both glucose and lactate levels are “safe” (euglycemia/eulactatemia); 2) elevated: both glucose and lactate levels are ±20% outside of normal levels (indicating beginning of a hyper/hypo glycemic or lactemic state); or 3) surgical LSI or death: both glucose and lactate levels are >20% outside of normal levels (indicating the patient is in a hypo/hyper glycemic or lactemic state (Figure 1). The windows of pathophysiological ranges were compiled from the literature. Normal glucose (3.88–5.5 mM) and normal lactate (0.5–1.0) levels indicated “safe,” whereas values outside that range (by 0–20%) indicated “elevated,” and levels far outside the normal range (>20%) indicated “surgical LSI or death.”[^32][33]

A data set of 150 samples was fabricated using randomly generated numbers defined to be within each class corresponding to the associated \(V_{AC}\) and \(V_{DC}\) (values were extracted from LabVIEW model) and verified during experimental evaluation of the biosensor. A 5% Gaussian white noise was added to each data set to simulate real measurements. Each data set was split into training (70%), validation (15%), and test (15%) sets. Performance of each neural network was measured by calculation of MSE of validation over training epochs, where the lowest MSE was defined to correspond to the scaled conjugate gradient algorithm (training ends when the MSE begins to increase). Statistical analysis of MSE’s was completed using Tukey’s test in JMP 14.0 (SAS Institute, Cary, NC, USA).

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest
The authors declare no conflict of interest.

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