Team Results Document
SenseNC

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SensUs 2022
Acute Inflammation with a focus on sepsis

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1. Abstract

Sepsis is a life-threatening condition characterized by systemic inflammation throughout the body due to infection, often resulting in septic shock (dangerously low blood pressure) and permanent organ damage if left untreated. This condition results in over 11 million deaths each year and is commonly diagnosed too late for treatment to be effective [1]. Within the United States, sepsis has been deemed one of the most expensive medical conditions to treat, with the cost of clinical intervention commonly ranging from $30,000 to $70,000 per patient [2]. Due to the high mortality rate of sepsis, it is important to develop a diagnostic tool capable of detecting sepsis at its early stages and tracking its severity and progression with high temporal resolution. The novel biosensor system we have developed aims to detect biomarkers of sepsis in human blood plasma and determine the severity of the condition. The target biomarker is the cytokine interleukin-6 (IL-6), a mediator of sepsis that plays a key role in the body's inflammatory immune response [3]. The system described herein uses monoclonal antibodies as a recognition element for IL-6 and a specialized electrochemical interface to perform electrochemical impedance spectroscopy (EIS) for quantification of IL-6 within a blood plasma sample. The final device was engineered to facilitate rapid, accurate, and resource-efficient detection of sepsis in a clinical setting.

2. Biosensor System and Assay

Overview. The biosensor system described herein utilizes a gold interdigitated electrode (IDE) functionalized with an anti-IL-6 antibody recognition element in order to detect IL-6 in blood plasma. The overall device usage and surface functionalization scheme of the biosensor is displayed in **Figure 1.** In principle, binding of IL-6 to the recognition element induces a change in measured sensor impedance, which is readily quantified using EIS. The magnitude and phase of impedance observed varies proportionally to the concentration of IL-6 present in the sample. Our team also investigated and developed a system to modulatel the pH of the sensing environment surrounding the recognition element, which is expected to control the biorecognition event between the recognition element and IL-6. This design may allow sensors to be reused and reduce the cost of quantifying IL-6 for the purpose of sepsis diagnosis. The system was designed to be a mobile point of care device, allowing nurses and emergency medical technicians (EMTs) to run tests using only an external computer and our miniaturized sensing platform.

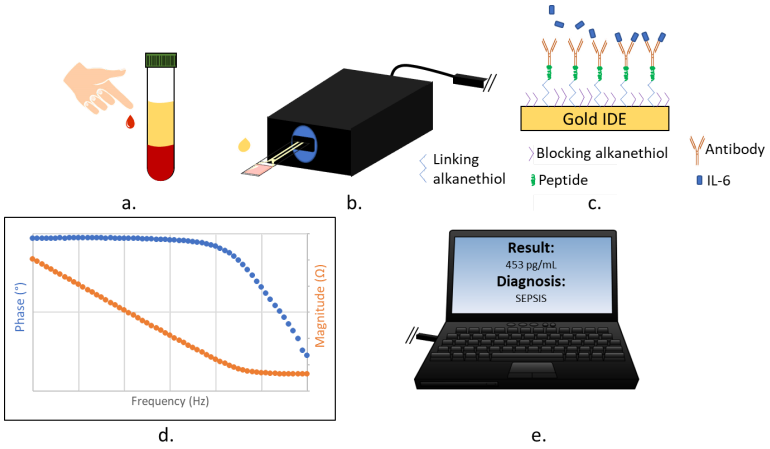


Figure 1. Operational Schematic. (a-b) Sample collection and application; (c) Binding of IL-6 to functionalized electrode; (d) Electrochemical impedance measurement; (e) Concentration calculation and diagnosis from computer software

Biorecognition and Reagents. The biorecognition element of the system is an immunoglobulin G1 (IgG1) monoclonal antibody (Ab) for IL-6. This antibody has been used throughout scientific research to investigate the physiological role that IL-6 plays within the body [4,5]. This antibody was commercially available and has been shown to exhibit high and specific affinity for IL-6 (588 pM [6]), making it ideal for the development of a biosensor designed to quantify the amount of IL-6 present in human blood plasma.

In order to immobilize the Abs onto the IDE, a mixture of alkanethiols was used to generate a self-assembled monolayer (SAM), which served two purposes: first, it provides the necessary surface chemistry to immobilize the recognition element onto the electrode surface, forming a bond between the gold of the IDE and the thiol functional group of the SAM; second, regions of the SAM that are not conjugated to the recognition element serve as a blocking agent, preventing surface fouling.

Immunoglobulin (IgG) Ab-based biosensors like the one described herein are receiving much attention for their vast potential in medical diagnostics [7]. SAMs are commonly utilized to immobilize such molecules, but the unpredictable nature of Ab orientation to SAMs causes variations in sensor surface chemistry and inconsistent electrochemical behavior [8]. ***To resolve this issue, a novel binding peptide (amino acid sequence: HWRGWVGK) was engineered to optimally link Abs to SAMs with consistently upright orientation.*** The peptide was designed such that the end terminal where IgG immobilization occurs is the fragment crystallization (Fc) receptor, which optimally binds with the Fc region of IgG molecules. As a result, this peptide can be used to immobilize almost any IgG recognition element [9], and is even applicable for fusion proteins. The surface chemistry deployed in this system rectifies a common issue in Ab-based biosensors and can be versatilely used in an abundance of biosensing applications.

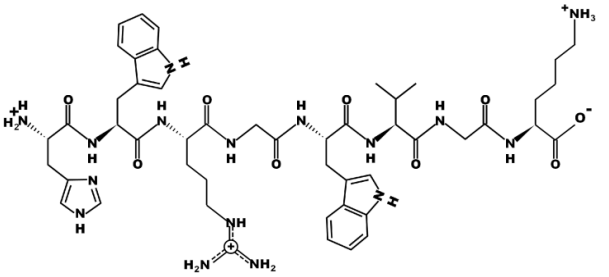


Figure 2. Chemical structure of the HWRGWVGK linking peptide.

Physical Transduction. Gold-on-glass IDEs were used for both the initial benchtop testing and the final integrated device. Prior to functionalization and electrochemical measurement, IDEs were cleaned by performing cyclic voltammetry in 0.5 M H_2SO_4 from -0.4 V to 1.4 V at a scan rate of 300 $mV \cdot s^{-1}$ for 15 cycles. After functionalization, a 14 µL sample containing IL-6 was pipetted into a fluidic chamber designed to contain a fixed sample volume, electrically connecting the two electrodes and completing the circuit. In order to determine the ideal frequency range in which a response to the presence of IL-6 was most readily observed, EIS was performed by applying a sine wave to the cell (10 mV RMS amplitude, 0 V DC offset) across a frequency range from 1 Hz to 1 MHz and measuring the resultant current. The surface chemistry of the electrode changed as IL-6 bound to the recognition element, producing a measurable change in the cell impedance.

A reference electrode (RE) was not used in the electrochemical cell. Both electrodes are functionalized and binding at either site contributes to the overall cell impedance. Since EIS only requires control of the whole cell potential, a RE is not needed. EIS is also capable of measuring non Faradaic current, in which no direct transfer of electrons occurs and the predominant contributor to electrochemical changes is due to capacitance [10]. This eliminates the need for a reduction or oxidation mediator and reduces the number of reagents necessary. Furthermore, fully functionalizing both electrodes of the IDE results in a larger area for binding to occur, which increases the observed effect of binding on impedance and improves the resolution of electrochemical measurements.

Cartridge Technology. To reduce device complexity, the cartridge technology was kept as simple as possible. The ideal diagnostic device minimizes the required patient sample volume. To achieve this, a small microfluidic cell was designed to hold 14 µL (0.45 mm x 7.6 mm x 4 mm, rounded edges)and was produced from PDMS to fit directly on top of the functionalized region of the IDE, as represented in **Figure 3**. The fluidic cartridge was then fixed on top of the IDE, allowing clinicians and medical professionals to easily load the patient sample (450 um x When blood plasma is applied to the microfluidic chamber, capillary action and surface tension result in a fixed volume being pulled onto the electrode surface and optimally spread out.

Figure 3. (a): Fluidic model displaying device structure. (b): Metrohm IDE. (c): Microfluidic chamber atop IDE. (d): Sample loaded into microfluidic chamber showing filling.

Mobile Instrumentation. The device's overall footprint needed to be minimized to ensure it could be deployed at the point of care and transported with ease. To ensure this requirement was met, the only instrumentation within the device itself is built around the AD5941 integrated circuit (Analog Devices) and performs the EIS measurement using code made by our team. The resultant data is then relayed to a computer, where further data processing and calculations provide the concentration of IL-6 in the patient sample using calibration data created during the development of the device.

User Interaction. Evaluating patients for sepsis will most commonly be performed by EMTs and nurses during patient transport and hospital evaluation, respectively. To accommodate for these clinical settings, our device simply plugs into the computers present in ambulances and mobile workstations through a USB port. This saves space in already confined environments and streamlines the process of incorporating such valuable prognostic information into electronic health records.

3. Technological Feasibility

Molecular Recognition. Initial investigations of the molecular recognition element began with verifying the high reported binding affinity between IL-6 and the anti-IL-6 antibody [4,11]. Surface plasmon resonance (SPR) was initially used to verify the reported binding affinity, but difficulties associated with the instrumentation used in SPR protocols prevented fruitful results from being produced. Limited quantitative verification was obtained to confirm the IL-6 antibody's binding affinity for IL-6, but the reported binding affinity in literature [4] and success of electrochemical measurements provided sufficient evidence that the recognition scheme was technologically feasible.

A brief experiment also evaluated the potential utility of the body's natural IL-6 signaling mechanism, which relies on the transmembrane cytokine receptor glycoprotein-130 (gp130) and the soluble IL-6 receptor (sIL-6R). Gp130 was not thoroughly assessed as a recognition element for the developed system due to economic infeasibility and increased reagent complexity, but preliminary results indicated a high binding affinity for IL-6 dependent upon the presence of sIL-6R within the tested sample [12]. Results from this analysis can be viewed in **Figure A1** of the appendix. These findings suggest that further research should be conducted on gp130's potential as a recognition element for the quantification of IL-6 and diagnosis of sepsis.

Electrode Functionalization. Electrodes were first cleaned using cyclic voltammetry as previously described. A SAM was then formed on the IDE surface by incubating IDEs in a mix of hydroxyl-terminated (2.45 µL of HS-C_{11}-EG_3-OH) and carboxyl-terminated (1.57 µL of HS-C_{11}-EG_6-OCH_2-COOH) alkanethiols in 10 mL of 100% ethanol in dark conditions. After SAM formation, electrodes were rinsed in 100% ethanol and dried under nitrogen gas. A solution of 200 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 50 mM N-hydroxysuccinimide (NHS) was then prepared in 1X phosphate buffered saline (PBS) and pipetted onto the surface of the electrode to activate the SAM. This reaction was allowed to proceed for one hour and was protected from light. The HWRGWVGK peptide was then conjugated to the activated surface by pipetting peptide solution (2mg/mL in 1X PBS) onto the electrode and incubating in darkness for six hours. Activated alkanethiols that did not bind with the HWRGWVGK peptide were blocked using a 50 mM ethanolamine solution (30-minute incubation). After blocking, the electrodes were rinsed with PBS and an anti-IL-6 IgG solution (0.5 mg/mL in 1X PBS) was pipetted directly onto the entire IDE surface. 150 µL of reagent was required for each step of the functionalization process. While ellipsometry, microscopy, and ion mass spectroscopy verified the success of the functionalization process, our team identified potential challenges in storing functionalized electrodes without losing the accuracy of measurement.

Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS). The formation of the SAM and conjugation of the HWRGWVGK peptide were verified using ToF-SIMS. The peptide was immobilized to a PEG-thiol SAM on a gold SPR chip. The conversion of carboxylic acid groups of the SAM to NHS ester groups via EDC/NHS was investigated under 2 conditions, using either MES buffer or Milli Q water for the EDC/NHS solution. Signatures of histidine and tryptophan peaks (m/z 110 for His; m/z 130, 159, 170 for Trp) of the peptide and fragments from the ethylene glycol units of the alkanethiol (CH_3O^+ m/z 31, $C_2H_3O^+$ m/z 43, $C_2H_5O^+$ m/z 45, $C_3H_7O^+$ m/z 59) were used as reference to evaluate the SAM formation and presence of the peptide in SAM-functionalized samples. The results indicated that both the SAM and HWRGWVGK peptides were successfully incorporated onto the surface of the IDE, and MilliQ water provided higher intensity of the peptide signature when used for the EDC/NHS solution (**Figure A2**, appendix).

Physical Transduction. Upon verifying IDE functionalization, EIS data was collected across a broad range of IL-6 concentrations. In addition to testing the sensor within the physiological range (0-68 pM), a concentration of 20 nM was tested to determine the sensor's upper limit of detection. This metric is important in determining a device's ability to accommodate physiological variation from patient to patient. The circuit equivalent of the electrochemical cell and the predicted change in impedance upon

ligand binding can be viewed in **Figure 4**. R_s represents the inherent resistance of the sample. R_{ct}, the resistance to charge transfer, is negligible in this case because no reduction or oxidation occurred. The most readily measured change in the circuit upon ligand binding was the double layer capacitance, C_{dl}. Results of the EIS experiments, which verified the circuit model, are present in **Figure 5** in addition to the derived calibration curve. Potential areas for improvement of the physical transduction method are discussed on page 18 of the Appendix.

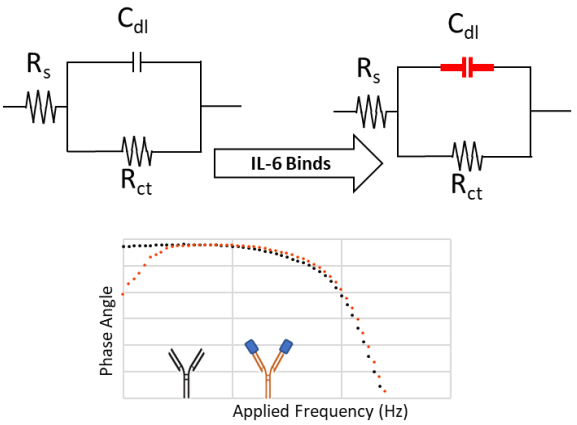
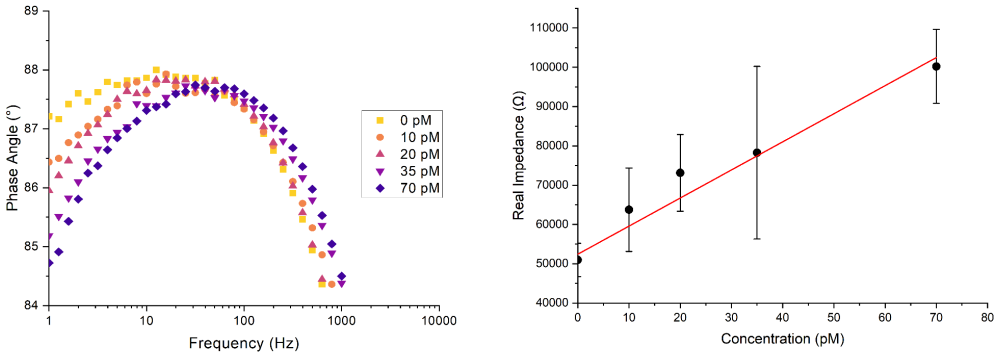


Figure 4. Randles circuit (circuit equivalent of electrochemical cell) and EIS Measurement before (left, black) and after (right, red) ligand binding. The predominant effect of binding was a change in measured capacitance.



a. **b.**

Figure 5. (a): Phase angle response to applied frequency for varying concentrations of IL-6. (b): Standard curve of real impedance vs. concentration of IL-6 derived from EIS measurements with a sensitivity of 715.24 Ohms·pM^{-1} of IL-6.

pH Modulation for Sensor Regeneration. Details about our efforts to develop a system to recycle biosensors by electronically modulating solution pH around the recognition element can be found on page 19 of the Appendix.

Fluidic Cartridge. The repeatability and reliability of sample loading using the microfluidic chamber were confirmed through a simple test in which 14 µL fluid samples were repetitively loaded into the sensor to verify containment and proper distribution of the sample. To validate inter-operator reliability, several individuals were tasked with loading samples into the fluidic cartridge, with all participants completing the task with minimal effort. It is worth noting that all participants in this test had experience with properly operating pipettes, but we expect most EMTs and nurses using our device to be familiar with such practices. The fluidic cartridge was designed to function similarly to a diabetic test strip. Given that the inspiration of the fluidic cartridge developed herein has had such a long history of technological reliability, the same can be cautiously expected of the fluidics used in our device.

Reader Instrument. The device's instrumentation relies primarily on off-the-shelf electronic components from well-established suppliers, ensuring reliable operation. EIS data generated using the AD5941 were compared to data collected using a commercial potentiostat (Metrohm) for verification. Diagnostic tests were repetitively performed using our device and various computers running the same software. No issues were reported, indicating that the system has few vulnerabilities that could result in device failure.

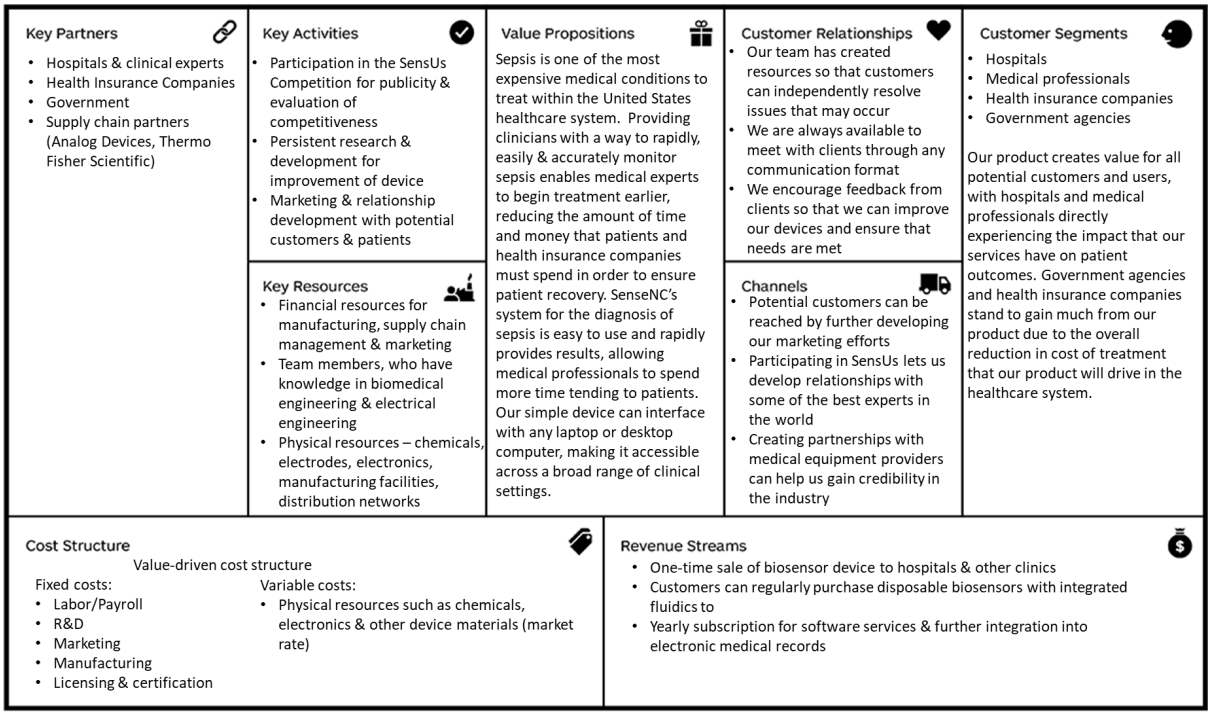
4. Originality

From the Team. SenseNC's approach to developing a biosensor for IL-6 in human blood plasma is novel in terms of both the chemical functionalization of the sensor and the electrochemical readout method employed. Our team was able to develop a repeatable process for functionalizing electrodes to bind with IL-6 present in liquid samples. The utilization of the HWRGWVGK peptide for consistent immobilization and orientation of IgG molecules in biosensors is original. This linking peptide should be further investigated for its potential in rectifying common issues associated with antibody-based biosensors. Once we were able to consistently functionalize electrodes for IL-6 detection, we determined that the complexity of the required electrochemical cell and sample handling system could be significantly reduced by using electrochemical impedance spectroscopy as our transduction method. To our knowledge, this electrochemical measurement technique has never been utilized to detect IL-6 in a repeatable manner. Furthermore, the system that we developed to generate a pH change for the regeneration of our recognition element to enable the reuse of these biosensors for multiple diagnostic tests is entirely novel. Although this system has not yet been fully incorporated into our final device, further work is being conducted by team members to achieve such integration. The team fully integrated the engineered biosensor with the measurement electronics and a user interface to develop a unique biosensing system.

From the Supervisor. The IL-6 immunosensor presented utilizes electrical impedance spectroscopy (EIS). In 2021, the first label-free electrochemical-based immunosensors for IL-6 were demonstrated. Typical IL-6 immunosensors have relied on either colorimetric or redox labeling to achieve necessary signal-to-noise ratio. By utilizing interdigitated electrodes and real-time adaptation and modeling of the Randles circuit, the team was able to demonstrate the initial improvement in sensor response and prediction of IL-6 concentration. Furthermore, the modification of electrode surface functionalization with a novel Fc-binding aptamer improved the display of the biorecognition element and made the strategy more generalizable to other biorecognition elements. All biochemical assays, electrochemical testing, hardware design, fabrication, and testing were carried out by the Team. Furthermore, testing to impThe Faculty Supervisors and Team Guides provided guidance and access to laboratory instrumentation and protocols, along with funds for material procurement. The team worked with their Faculty Supervisors to conceive the immunosensor and future regeneration strategies, and the Team independently carried out the research and development objectives. Testing of the electrochemical sensors, complete development of the electronic hardware, investigation of the translation potential, and completion of all SensUs requirements were completed nearly 100% independently by the members of the Team.

5. Translation potential

Business Model Canvas



Stakeholder Desirability.

Approximately 48.9 million cases of sepsis occurred worldwide in 2017 [1]. Globally, nearly 11 million deaths occur each year due to sepsis, which equates to one person dying of sepsis every 2.8 seconds [1]. Sepsis has been deemed one of the most expensive conditions to treat on an inpatient basis in the United States, placing a heavy financial burden on health insurance companies, as well as patients and their families [2]. Within a hospital, nearly 1 in every 3 deaths that occur is caused by sepsis [1]. Currently, detection of suspected cases of this deadly condition is primarily accomplished through monitoring of physiological signs such as a patient's body temperature, the recent history of infection, and the presence of extreme illness [13]. Suspected cases are definitively confirmed through blood culture techniques, which, while highly accurate, require substantial processing time. In the context of sepsis, delays in the provision of appropriate care are severely detrimental to patient outcomes. Through our interviews with medical professionals at the Brody School of Medicine at East Carolina University, we have confirmed that treatment of sepsis commonly relies on the administration of antibiotics, intravenous fluids, and in extreme cases, surgical intervention to remove infected tissue.

The burden of sepsis is felt heavily throughout the world due to its high mortality rate and substantial financial burden. SenseNC strongly believes that the tragic outcomes of this disease can be dramatically reduced by developing and distributing systems with the capability of diagnosing and monitoring sepsis at its earliest stages, rather than being forced to wait until disease progression produces obvious outward signs. Due to the astronomical costs associated with the treatment of sepsis in the United States (often approaching $70,000 for treatment of one patient in severe cases [2]) and the physical suffering endured by victims of the disease, we believe that the diagnostic system described herein has the potential to vastly improve patient outcomes and reduce the cost of treatment by providing medical professionals with the tools necessary to proactively treat sepsis in the earliest stages of its progression.

Business Feasibility.

While the overall business model presented in the Business Model Canvas is entirely feasible, further development of the biosensor system is necessary if we wish to meet the level of accuracy and consistency required by medical professionals in order to assist them in making appropriate medical decisions. With the current size of our team, the amount of research necessary to develop our biosensor to clinical standards would take several years. Therefore, in order to expedite the development process, an expansion of our research team is necessary. This expansion should also diversify the expertise of our team so that we have technical knowledge in the realms of software engineering and scalable manufacturing. Specifically, our device needs to be further optimized by calibrating our electrochemical measurement scheme against external sources of error such as ambient temperature and humidity and further enhancing our fabrication methods such that our sensors are more robust to the unpredictable shipment conditions and storage.

It is also important that the device's reliability be thoroughly tested in a clinical setting by evaluating the accuracy of the system with blood samples of patients susceptible to sepsis, such as elderly individuals and those with chronic illnesses [2]. These demographics are expected to have elevated levels of IL-6 in their blood. Whether or not the device is able to correctly identify abnormally high levels of IL-6 in these individuals in comparison to the general population would provide an excellent indication of the device's clinical efficacy.

The field of medical diagnostics moves at a very fast pace. Members of our team need to continually reevaluate the state-of-the-art sepsis diagnostic techniques to ensure that our technology remains globally competitive and medically relevant. This must be done by continuously researching the best options for sepsis diagnostics and maintaining an open dialogue with medical professionals. Our entrepreneurial pursuits will also be significantly aided by pursuing partnerships with critical suppliers such as Analog Devices, Thermo Fisher, and Metrohm. We will have much more to offer in such partnerships once we have perfected our biosensor and can offer these companies opportunities for brand exposure and reliable business.

It is vital that we work to leverage economies of scale in our manufacturing process as we expand our market share and the role that our device plays in the treatment of sepsis. This will enable us to reduce the financial cost associated with production, allowing our team to prioritize the accessibility of our devices and the impact that they have on patient well-being. Such business developments would make it more practical for clinicians to track disease progression with multiple tests, likely resulting in more effective treatment.

Financial Viability.

The cost-effectiveness of our proposed business model is primarily based on the financial resources that hospitals, health insurance companies, and patients could potentially save by investing in our products and services throughout the United States. While the overall prototyping cost of our device and sensor was approximately $500, this price could be significantly reduced by simplifying the electronic components of the device using custom printed circuit boards, using injection molding rather than 3D printing for the device enclosure, and increasing the quantities of chemicals purchased for production [14]. We believe that the development of a large-scale manufacturing process and scalable supply chain could reduce the cost associated with each set of sensing electronics to a more reasonable cost of $200 and a sensor cost of approximately $15. By pricing our products using a value-based model, it is reasonable to set the price of our device at $500 and each sensor at $30. These prices are marked up to account for the costs associated with sterile manufacturing techniques, labor, marketing, and distribution. Within the United States, this price is of excellent value for a critical diagnostic device.

The anticipated market size for our products and services is split amongst the one-time sale of our electronic device and recurring sales of our biosensor. In the early stages of business development, our efforts would be entirely focused on capturing market share within the United States. There is currently no clear competitive edge in the sepsis diagnostic market, indicating that upon fully developing our technology to clinical standards, it is reasonable to optimistically estimate

that we would be able to capture 10% of the United States market due to technological superiority and superb customer service. Given that there are approximately 6,100 hospitals in the United States [15], selling just one device to 10% of these hospitals would result in one-time revenue of $304,650. If 10% of the 170,000 patients in the United States that develop sepsis annually were to rely on our biosensor for just one diagnostic test, annual revenue would be approximately $5,100,000. We also hope to incorporate our pH modulation-based biosensor regeneration system into this device, as described on page 19 of the appendix. While providing this system to customers would reduce the revenue generated from biosensor sales, it would vastly improve the affordability of our system, which would likely result in a larger share of the market. Given these projections, the current pricing of our business model is financially sustainable.

6. Team and support

Dr. Michael Daniele and Dr. Stefano Menegatti:

Michael Daniele and Stefano Menegatti are the team's supervisors. They provided guidance on technical decisions, instruction, and resources for team members to gain skills and knowledge.

Kaila Peterson and Jack Twiddy:

Kaila Peterson and Jack Twiddy are team coaches and assisted team members throughout the development of the biosensor by providing technical support for electrochemical testing and sensor fabrication, as well as peer review.

Angelica Aroche:

Angelica Aroche is a member of the chemistry team. She is responsible for sensor fabrication and electrochemical measurements. She also made major contributions to surface chemistry verification, overseeing surface plasmon resonance, and secondary ion mass spectroscopy.

Christopher Sharkey:

Christopher Sharkey is a member of the chemistry team. He is responsible for sensor fabrication, electrochemical measurements, and fluidics. He also leads continuing research into the team's pH control system and led the development of the team's business model.

Leslie Uy:

Leslie Uy is a member of the chemistry team. She is responsible for sensor fabrication and surface plasmon resonance experiments. She also made contributions to the team's social media presence and marketing efforts.

Dhruv Sadhu:

Dhruv Sadhu is a member of the electronics team. He contributes to the design and testing of electronic hardware that performs electrochemical impedance spectroscopy within the device. He also led interviews with medical professionals.

Catherine Denisowski:

Catherine Denisowski is a member of the electronics team. She contributes to the design and testing of electronic hardware that performs electrochemical impedance spectroscopy within the device.

7. Final Remarks

While the current biosensor shows promise for the detection of interleukin-6, we intend to continue our research efforts to improve the accuracy and dynamic range of our device. Throughout this process, the team has learned a considerable amount about the development of biosensors and many of us intend to continue similar work within the field of medical diagnostics throughout our careers.

As previously discussed, preliminary data have shown that gp130 may be an excellent biorecognition element for IL-6. Some of our research efforts going forward will be focused on verifying this recognition element's utility in sepsis diagnostics and investigating methods of making such a recognition scheme economically feasible.

The team members would like to express gratitude to our mentors and coaches who have helped us throughout this process. We are grateful for the opportunity to participate in the SensUs Student Competition and everything we have learned throughout this process. This work was also made possible through support from North Carolina State University's Office of Undergraduate Research, Engineer Your Experience Program, and the National Science Foundation.

8. References

[1] World Health Organization. “Sepsis.” (August 26, 2020). https://www.who.int/news-room/fact-sheets/detail/sepsis

[2] Paoli, C., Reynolds, M., Sinha, M., Gitlin, M., & Crouser, E. “Epidemiology and Costs of Sepsis in the United States—An Analysis Based on Timing of Diagnosis and Severity Level.” Critical Care Medicine, 46(12), 1889-1897 (2018). https://doi.org/10.1097%2FCCM.0000000000003342

[3] Song, Juhun, Dae Won Park, Sungwoo Moon, Han-Jin Cho, Jong Hak Park, Hyeri Seok and Won Seok Choi. “Diagnostic and Prognostic Value of Interleukin-6, Pentraxin 3, and Procalcitonin Levels among Sepsis and Septic Shock Patients: A Prospective Controlled Study According to the Sepsis-3 Definitions.” BMC Infectious Diseases 19, no. 968 (November 12, 2019): https://doi.org/10.1186/s12879-019-4618-7

[4] Worsley, Graham, Neelam Kumarswami, Caterina Minelli and James E. Noble. “Characterisation of Antibody Conjugated Particles and their Influence on Diagnostic Assay Response.” Analytical Methods, no. 7 (October 13th, 2015): 9596-9603. https://pubs.rsc.org/en/content/articlelanding/2015/AY/C5AY01775K

[5] Heremins, Hubertine, Chris Dillen, Willy Put, Jo Van Damme and Alfons Billiau. “Protective Effect of Anti-interleukin (IL)-6 Antibody Against Endotoxin, Associated with Paradoxically Increased IL-6 Levels.” European Journal of Immunology 22, no. 9 (September 1992): https://doi.org/10.1002/eji.1830220932

[6] Wang, Chun-Wei and Rakesh Kapoor. "Dissociation Constant Measurement Using Combination Tapered Fiber-optic Biosensor (CTFOB) Dip-probes." Proc SPIE Int Soc Opt Eng (2010). https://doi.org/10.1117/12.843720

[7] Conroy, Paul, Stephen Hearty, Paul Leonard and Richard J. O'Kennedy. "Antibody Production, Design and Use for Biosensor-based Applications." Seminars in Cell & Developmental Biology 20, no. 1 (February 2009): https://doi.org/10.1016/j.semcdb.2009.01.010

[8] Makaraviciute, Asta and Almira Ramanaviciene. "Site-directed Antibody Immobilization Techniques for Immunosensors." Biosensors and Bioelectronics 50, (December 15th 2013): https://doi.org/10.1016/j.bios.2013.06.060

[9] Davies, David R. and Susan Chacko. "Antibody Structure." Accounts of Chemical Research 26, no. 8 (August 1st, 1993): https://doi.org/10.1021/ar00032a005

[10] Lasia, Andrzej. “Electrochemical Impedance Spectroscopy and its Applications.” Modern Aspects of Electrochemistry 32, (1999): 2395-2401. https://doi.org/10.1007/0-306-46916-2_2

[11] Alfinito, Eleonora, Matteo Beccaria and Mariangela Ciccarese. “Biosensing Cytokine IL-6: A Comparative Analysis of Natural and Synthetic Receptors.” Biosensors 10, no. 9 (August 24th, 2020). https://www.mdpi.com/2079-6374/10/9/106

[12] Boulanger, M. J., Chow, D. C., Brevnova, E. E., & Garcia, K. C. (2003). “Hexameric structure and assembly of the interleukin-6/IL-6 alpha-receptor/gp130 complex.” Science (New York, N.Y.), 300(5628), 2101–2104. https://doi.org/10.1126/science.1083901

[13] Hunt, Anne. “Sepsis: An Overview of the Signs, Symptoms, Diagnosis, Treatment and Pathophysiology.” Emergency Nurse 27, no. 5 (September 2nd, 2019): 32-41. https://pubmed.ncbi.nlm.nih.gov/31475503/

[14] Franchetti, Matthew and Connor Kress. “An Economic Analysis Comparing the Cost Feasibility of Replacing Injection Molding Processes with Emerging Additive Manufacturing Techniques.” The International Journal of Advanced Manufacturing Technology 88, (June 3rd, 2016): 2573-2579. https://doi.org/10.1007/s00170-016-8968-7

[15] “Fast Facts on U.S. Hospitals, 2022: AHA.” *American Hospital Association*, https://www.aha.org/statistics/fast-facts-us-hospitals.

9. Appendix

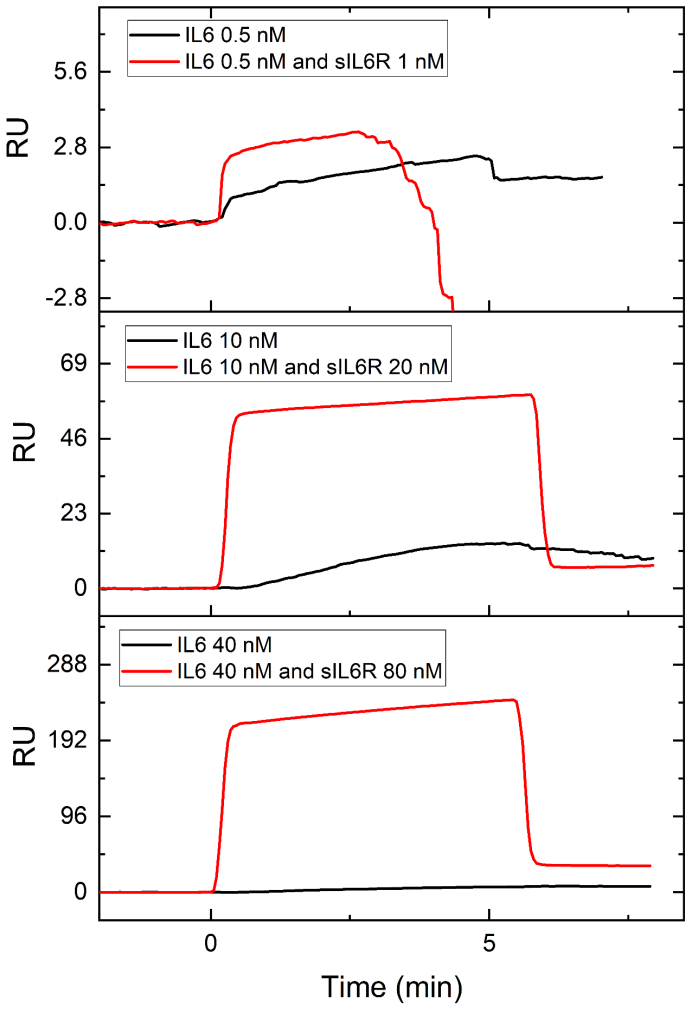


Figure A1. SPR sensorgrams with gp130 immobilized on a gold chip. Results indicate that the binding affinity of gp130 for IL-6 is extremely high, but dependent on the presence of sIL-6R.

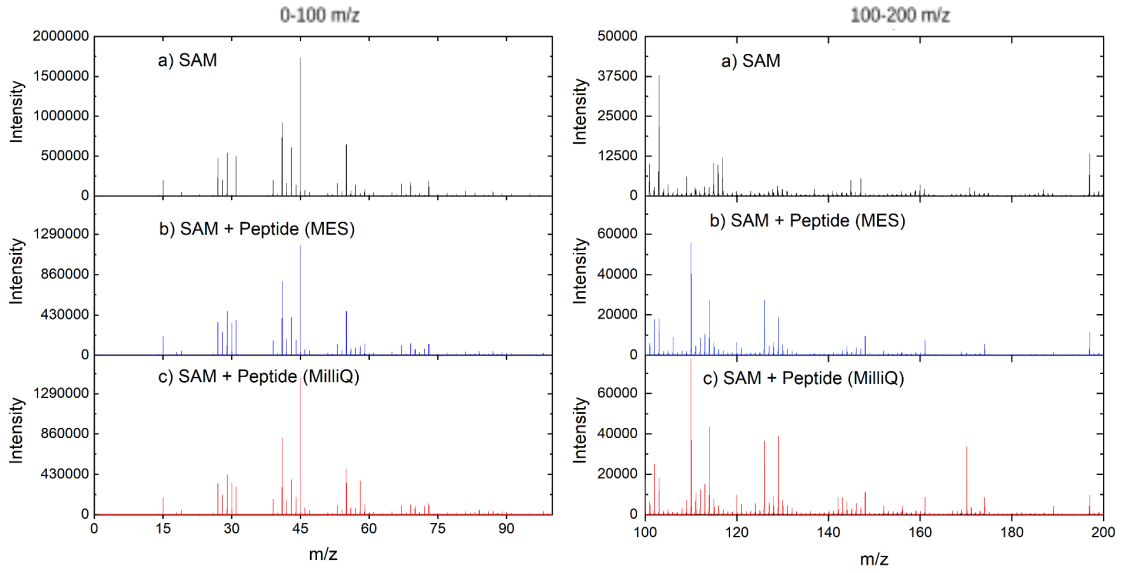


Figure A2. ToF-SIMS spectra within the range of 0-100 m/z (left) and 100-200 m/z (right) of a) SAM on gold; b) HWRGWVGK peptide covalently attached to SAM using MES buffer (2-(N-morpholino)ethanesulfonic acid) in the EDC/NHS activation; c) HWRGWVGK peptide covalently attached to SAM using MilliQ water in the EDC/NHS activation. Separate buffers were tested to determine optimal solution for immobilization of SAM.

Potential Areas for Improvement of Physical Transduction Methods

Our biosensor was designed to selectively bind IL-6 and measure the concentration of IL-6 in blood plasma, which makes it a potentially useful tool in diagnosing and monitoring acute inflammation and sepsis. However, due to financial constraints, all electrochemical measurements performed during the development of our calibration curve used IL-6 in 1X PBS, rather than the more clinically relevant human blood plasma. The presence of additional biochemicals in tested samples could introduce large amounts of electrochemical noise to the system, rendering calculations of IL-6 concentrations inaccurate. We attempted to avoid this issue by fully coating electrodes in alkanethiols and blocking alkanethiols that did not form complexes with our selected biorecognition element (the anti-IL-6 antibody). In theory, this should drastically reduce the biosensor's reactivity to biomolecules within blood plasma other than the analyte of interest. However, this has not yet been experimentally verified. Physical transduction and resultant calculations of target ligand concentration could likely be made more accurate by incorporating electrochemical data collected with samples containing interferent species in addition to IL-6. Furthermore, despite its accuracy, electrochemical impedance spectroscopy is known to be a rather delicate measurement technique. The presence of electromagnetic interference (EMI), abnormal ambient temperatures and slight deviations of electrode geometry can alter the measured impedance of the biosensor's electrochemical cell, resulting in inaccurate measurement of the target ligand[1]. This problem can be mitigated by incorporating these external variables into the calibration process of the diagnostic tool. The effect of these sources of interference can be minimized through the addition of other engineering controls to the device itself (e.g., temperature controls, vibrational shielding, etc.). Additionally, the incorporation of a Faraday cage around the sensor can reduce the effect of EMI on sensor accuracy. Further investigation into potential sources of error in EIS measurements must be investigated and addressed in order to ensure consistent electrochemical measurements using this technique.

[1] Talian, Sara, Joze Moskon, Robert Dominko, Miran Gaberscek. "The Pitfalls and Opportunities of Impedance Spectroscopy in Lithium Sulfur Batteries." Advanced Materials Interface 9, no. 8 (November 15th, 2021). https://doi.org/10.1002/admi.202101116

pH Modulation for Recycling of Antibodies. In tandem with our work in developing a biosensor for IL-6, our team developed a system for electronically controlling the pH of an electrochemical cell in order to reverse binding between the biorecognition element and target analyte. The system was developed using palladium as a working electrode due to its voltage-dependent ability to absorb and release hydrogen ions, which directly influences the cell's pH. The affinity that a recognition element expresses for a target analyte is typically pH-dependent. Changes in pH can induce denaturations in either element of the biorecognition system (the recognition element or the analyte itself), resulting in unbinding. If this phenomenon is adequately controlled within *in vitro* diagnostic systems, it is theoretically possible to regenerate a biorecognition element's ability to capture the target analyte and thus reuse the same functionalized electrodes for multiple diagnostic tests. An overview of the system can be viewed in **Figure A3**. Although this system was not incorporated into our final device due to time constraints, initial experimental results indicate that such a system is able to adequately modulate a cell's pH and reverse binding between biotin and streptavidin, a pair known to exhibit extremely high binding affinity[2]. Results of monitoring the pH of an electrochemical cell while applying a square wave potential of varying amplitudes is shown in **Figure A4**. Furthermore, fluorescence microscopy images of biotinylated glass slides that were exposed to fluorescently-labeled streptavidin at neutral pH (favorable for binding) and then exposed to highly basic pH (unfavorable for binding) are also present, showing a clear decrease in the overall observed fluorescence (indicative of a low amount of bound streptavidin) as the pH was modulated using our system (**Figure A5**). Ongoing efforts will aim to display this system's utility in regenerable biosensing applications.

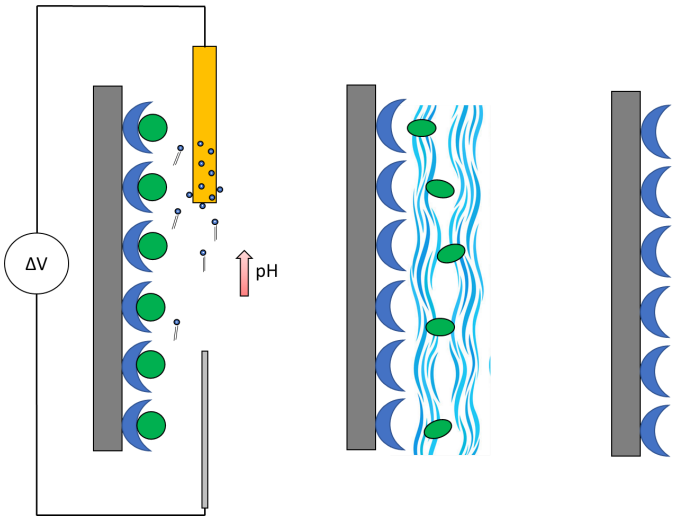


Figure A3. Overview of pH control system, displaying (left): Application of an electrical potential to Palladium with respect to Ag/AgCl reference inducing absorption and adsorption of hydrogen ions, resulting in a localized increase in pH around the saturated biorecognition element; (middle): Conformational changes to the biorecognition element and ligand causing unbinding and flushing with a buffer causing the ligand to be removed from the electrochemical cell; (right): Regenerated biosensor ready to capture target analyte in another patient sample.

[2] Weber, Patricia, D. H. Ohlendorf, J. J. Wendoloski and F. R. Salemme. "Structural Origins of High-Affinity Biotin Binding to Streptavidin." Science 243, no. 4887 (January 6th, 1989): https://www.science.org/doi/10.1126/science.2911722

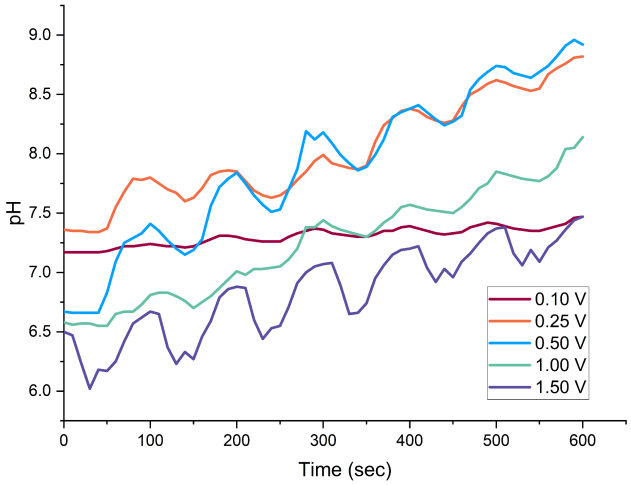


Figure A4. pH responses recorded as square wave voltammetric waveforms of varying amplitudes were applied to the electronic pH control system. All applied waveforms were centered around 0 V.

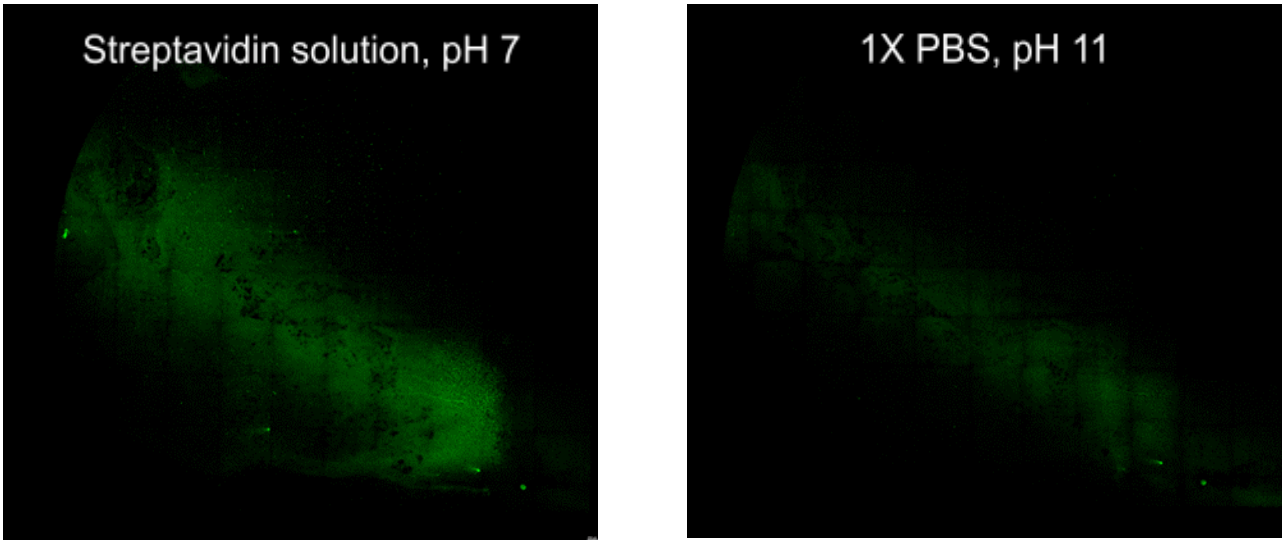


Figure A5. Left: Biotinylated glass slides exposed to fluorescently-labeled streptavidin at a pH of 7.
Right: Same slide exposed to solution of 1X PBS at a pH of 11.
In both cases, pH levels were achieved using the described system