Team Results Document
SenseNC

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SensUs 2023
Traumatic Brain Injury

SensUs

1. Abstract

Traumatic brain injury (TBI) is clinically defined as a condition where the brain sustains an abrupt external insult. Depending on the severity of the injury, it can affect a wide range of cognitive and motor functions such as memory loss and poor coordination. Currently TBI is diagnosed by initially monitoring the patient's physical and psychological state and then determining the need for a CT scan. However, CT scans are expensive, involve exposure to radiation, and are not reliable due to obscured views of some brain regions. A possible solution is the use of glial fibrillary acidic protein (GFAP) as a biomarker of TBI. Once a TBI has occurred, astrocytes are activated, neurotoxins are released and there is an increase in GFAP in the bloodstream. Our device functions as an organic electrochemical transistor (OECT) that produces measurable electrical changes in response to antigen (GFAP) binding to the functionalized working electrode. The final product is a portable point-of-care test device that rapidly measures and displays the severity of a TBI.

2. Biosensor system and assay

2.1. Device Overview

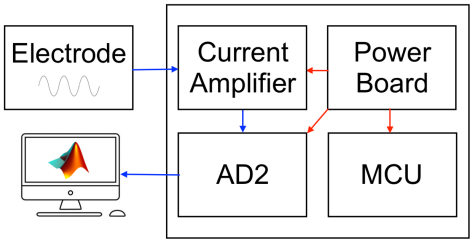


Fig. 1

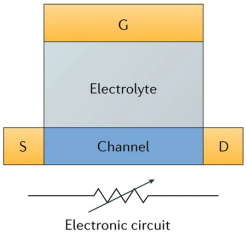


Fig. 2 [1]

Fig. 3

Our electrode functions as an organic electrochemical transistor (OECT) which transduces GFAP binding through changes in channel current to detect clinically relevant (0.01-10 ng/mL) levels of GFAP. An OECT is made of a gate, drain, and source, with an ion-permeable channel between the drain and source, and an electrolyte which separates the gate from the channel (fig. 2). In this case, the gate is functionalized to bind GFAP. When a voltage is applied across the source and drain, there is a channel current response which changes based on the effective voltage of the gate. The effective voltage of the gate changes as GFAP binds.

The channel current is very small (nA-μA), so an external circuit amplifies channel current into an easily measurable level. A printed circuit board (PCB) was designed and fabricated to accomplish this (section 2.5).

The Analog Discovery 2 (Digilent) is a USB oscilloscope and multi-function instrument which can perform the precise measurement, recording, and generation of voltage signals in a small form factor (fig. 3). This device was utilized to measure our current amplifier output and provide the drain-source and reference voltage to measure our OECT.

A power subsystem was designed for our device, which uses a PCB to produce voltage rails for all the components from a single micro-USB connection. This also communicates with the AD2 (section 4).

A graphical user interface (GUI) was developed in order to abstract the end-user from the device's hardware. Through an app written in MATLAB (MathWorks), users can manipulate the drain-source voltage, reference voltage, sampling parameters, and view the biosensor's test results (section 5).

2.2. Molecular Recognition and Assay Reagents

The proposed sensor system, shown in figure 4, works as an off-sensor modality, which results in a decrease in output signal as the analyte concentration increases. Current state of the art sensor platforms often encounter sensitivity issues, such as poor limits of detection based on the transduction limits of the utilized equipment and/or the feasibility of the effectiveness of functionalization. In an attempt to mitigate these challenges, OECT technology was implemented. One of the many advantages of this technology is the enhanced current signal that can be obtained.

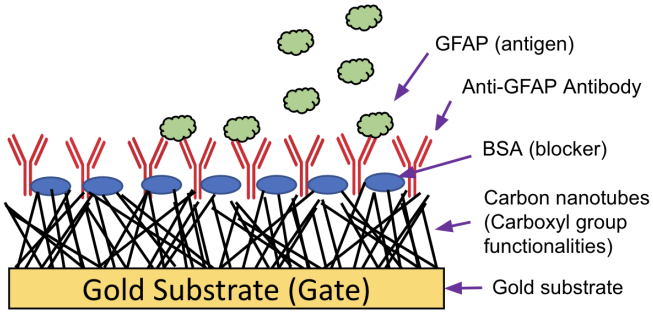


Fig. 4

The sensor is composed of a commercially available electrode, which was modified with a 5 mm diameter film of carbon nanotubes (CNTs) on the working electrode (OECT gate). The CNTs have carboxylic acid moieties, which enable further functionalization through bioconjugation with the receptor/antibody's residual amine groups. Using CNTs not only facilitates functionalization, but also provides an enlarged surface area and enhanced current response, which mitigates the most common problems faced in similar platforms. The OECT's channel is composed of a printable (poly(3,4-ethylenedioxythiophene) polystyrene sulfonate) (PEDOT:PSS) ink modified with poly(ethylene glycol) diglycydil ether (PEGDE) for enhanced stability.

OECT immobilization was adapted from Lee et. al [2], and consists of three major steps. The first step activates the carboxylic acid moieties, employing the application of EDC and NHS, which functions by carbodiimide crosslinking between the surface and bioreceptors (antibodies) [3]. This is followed by the addition of antibodies. The solution is drop casted and incubated for two hours. The final step is the addition of a backfiller; in this case, BSA. Its purpose is to block any exposed CNT

surface and unbound carboxylic acid moieties as a means of preventing non-specific binding, which enhances the system's selectivity.

2.3. Physical Transduction

The sensor system utilizes electrochemical transduction. The quantity of GFAP binding on the functionalized gate modulates the surface potential of the gate within the electrochemical cell. The channel is composed of an ion-permeable material and the permeability to ions changes in response to the effective voltage of the gate. Consequently, channel current changes as GFAP binds to the gate. This current shift is a parameter that can be quantitatively assessed through electrochemical measurements utilizing the developed electronics. The measured current is correlated to the concentration of GFAP within the sample, providing a means to determine and assess GFAP concentration levels using this system.

2.4. Cartridge Technology

Our sensor does not require sample processing prior to placing the sample on the sensor. We apply a molded PDMS cap to the surface of the sensor, which creates a reservoir for the sample fluid around the channel and gate of the OECT. The reservoir allows for a pipette tip to be inserted and can hold 20µL of sample fluid. The PDMS cap is reusable and can be rinsed clean for future use after the sample has been measured. The sensor itself and the sample fluid are disposed of after use.

2.5. Reader Instrument and User Interaction

Our sensor uses a commercial off the shelf connector that allows the electrode to be connected to the hardware. To use the sensor system, the user first inserts a new sensor into the connector. The connector will be modified to give visual aids for placement of the electrode. The user would then place the PDMS cap onto the sensor, ensuring that the reservoir surrounds the gate and channel of the OECT. The PDMS cap is directionally designed and specifically dimensioned to minimize user error. Once the PDMS cap is in place, the user would insert a pipette into the reservoir and deposit the sample. In the future, a single volume dropper could be included in packaging to minimize user error and increase user-friendliness.

2.5.1 Hardware

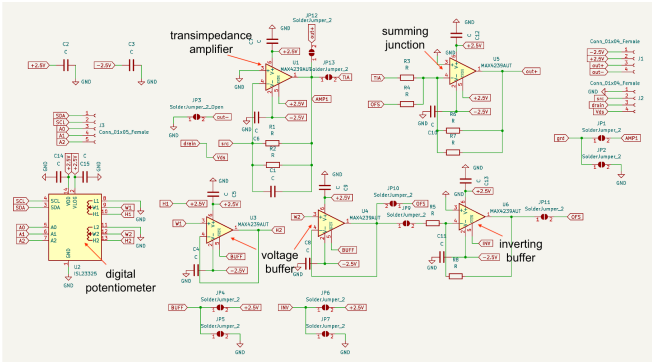


Fig. 5

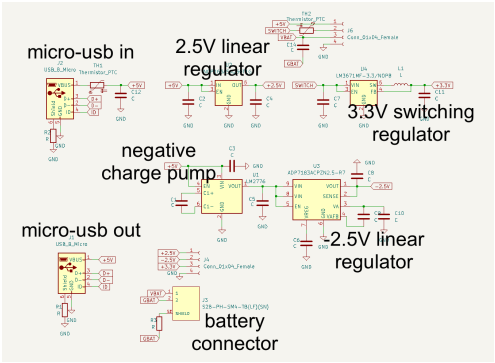
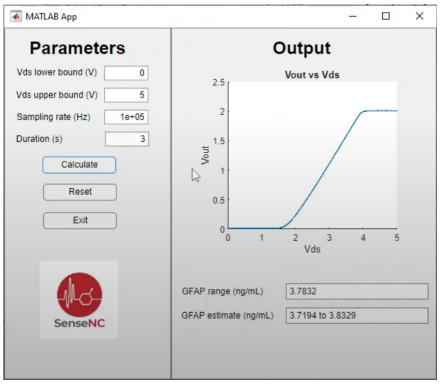


Fig. 6

Fig. 5: Circuit schematic for the two-stage current-to-voltage amplification circuit we designed. The channel current is fed into a transimpedance amplifier (TIA) which amplifies the current to a voltage proportional to the feedback resistance. The TIA output goes into a summing junction with a voltage correction which removes the offset from the first stage of amplification, and the summing junction acts as a second stage of amplification. Fig. 6: Circuit schematic for the power subsystem. The PCB takes a micro-usb connection and uses voltage regulators to generate all the voltage rails needed to power the current amplifier, microcontroller, and AD2. It also has polymeric positive temperature coefficient (PTC) fuses for short circuit protection.



2.5.2 Software

Using the interface we developed in MATLAB, Fig. 7, users will type into text boxes to change the reference voltage, drain-source voltage, sampling rate, and duration of data collection. Using 'calculate' displays the output voltage from the amplification board, a GFAP range, and a GFAP estimate.

Fig. 7

3. Technological feasibility

3.1 Sensor Fabrication and Functionality

There are three main portions of the sensor that required specific development in order to create the OECT: the conductive polymer channel (i.e., PEDOT:PSS), the CNT film, and the antibody immobilization process.

PEDOT:PSS is a water-soluble polymer that we placed on the OECT channel to act as an organic semiconductor. However, during initial testing, as the sample fluid passed over the PEDOT:PSS channel on its way to the working electrode, the channel was stripped from the electrode. To overcome this challenge, we modified the PEDOT:PSS by adding PEGDE. The carbon chains present in PEGDE inhibit hydrogen bonding, which causes the PEGDE-PEDOT:PSS molecule to fold in on itself [4]. This folding "blocks" the moieties in PEDOT:PSS that cause its hydrophilicity, which allows the PEDOT:PSS to remain on the electrode while submerged in liquid. This is not only important for applying the sample to the sensor, but also ensuring the sensor is stable.

Carbon nanotubes were chosen for the surface modification of the gate as an alternative to traditional self-assembled monolayers for multiple reasons. First, the increased surface area obtained by adding a CNT film to the gate results in amplified current signals, which correlates to an exponential increase in sensitivity. We also chose multiwalled CNTs (MWCNT) with carboxylic acid functional groups so that anti-GFAP antibodies could be bound directly to the CNTs via EDC/NHS chemistry. We specifically chose MWCNTs because they are more cost effective than single-walled CNTs and they function as desired since the carboxylic acid functional groups protrude from the exterior of the MWCNT. The two main challenges encountered while depositing CNTs onto our sensor were creating a fully dispersed solution of CNTs and adhering the CNTs to the sensor. We first attempted to directly pipette the CNT dispersion onto the gate of the sensor. With this approach, the results ranged from an uneven gray coloration to clumps of CNTs irregularly patterned. The variability of the CNTs on the gate led to inaccurate transduction. We used multiple concentrations of CNTs in our initial dispersions and used both bath sonication and probe sonication to try to fully disperse the solution. After these failed attempts at directly dropping the CNT dispersion onto the gate, we dove back into the literature and decided to shift to developing CNT films. Our process for creating CNT films was adapted from Palomar et al [5]. We created a dispersion, sonicated, and let the solution incubate for 24 hours to let the non-dispersed CNTs settle. The supernatant was then filtered through cellulose paper via vacuum filtration. The combination of only using the supernatant and applying vacuum filtration resulted in uniform, reproducible CNT films. Upon achieving consistent film fabrication, CNTs were deposited onto sensors and functionalized using EDC/NHS chemistry with no additional cross linkers or intermediary reagents. The resulting correlation between GFAP concentration and channel current is present in Fig. 8. The results are linear and demonstrate a high correlation between GFAP concentration and measured current. Measurements were taken in blood plasma and demonstrated high specificity for the target analyte.

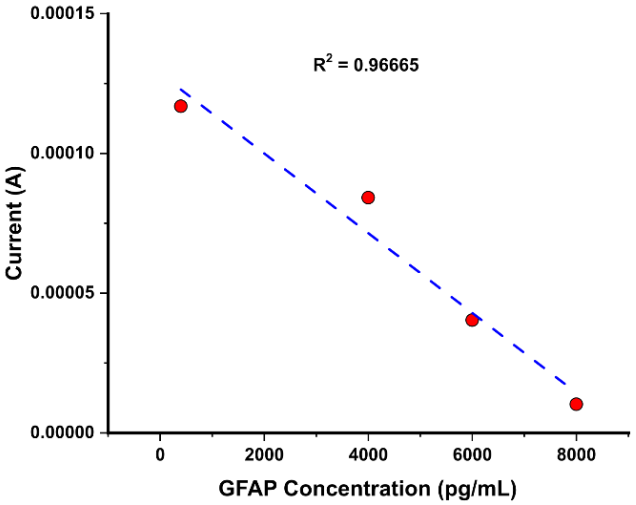


Fig. 8

3.2 Reader Instrument

3.2.1 Circuit Design Verification

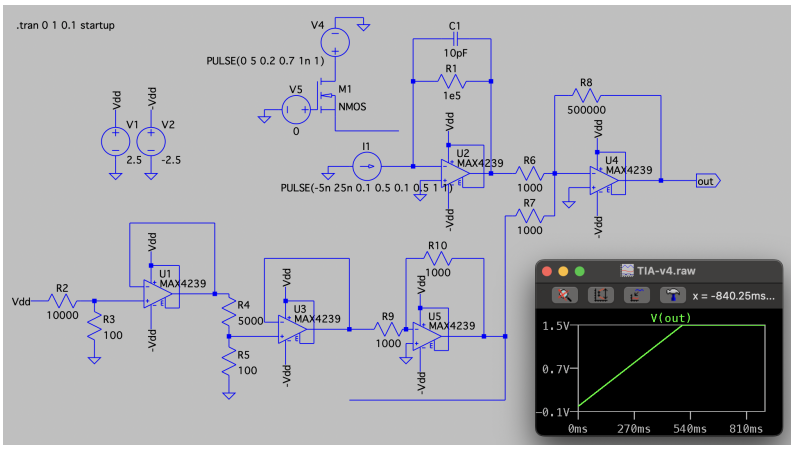


Fig. 9

Simulation software (LTSpice, Analog Devices), Fig. 9, was used to simulate our amplifier circuit before fabricating it as a PCB. We verified that the theoretical models of our circuits wcould amplify the relevant current range (nA-μA) with little voltage offset and high reproducibility.

3.2.2 Analog Circuit Verification

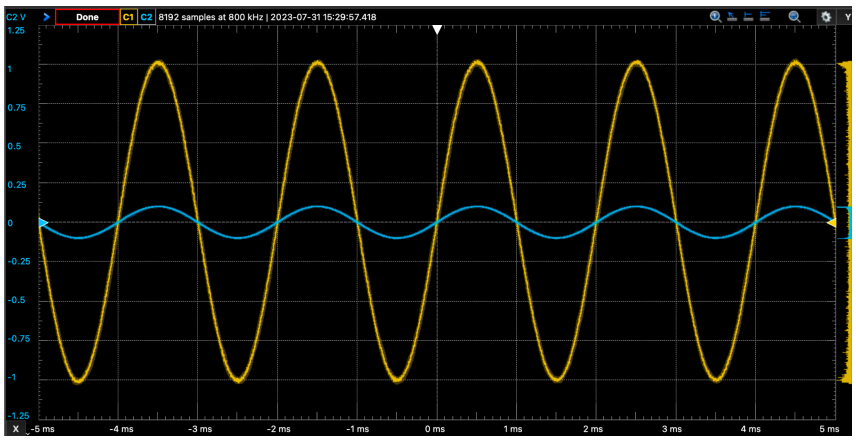


Fig. 10

We conducted isolated testing on our amplification and power circuit using the Analog Discovery 2 to verify the circuits are working as intended. The oscilloscope in Fig. 10 features a 100 mV sine wave (blue) fed into a 10 kΩ resistor resulting in a 1 uA signal which is expected to have 100,000x amplification based on feedback resistance. Our 1 uA current input amplified to the expected 1V output signal (orange) with less than 1 mV or 0.1% of offset error.

3.2.3 Software Testing

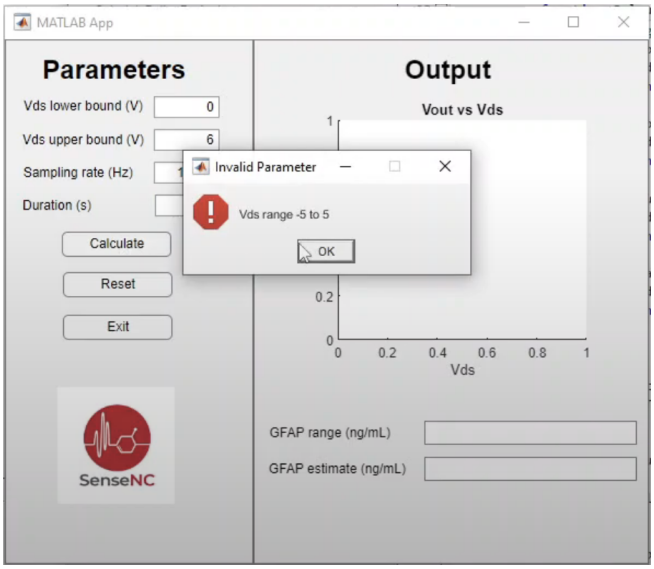


Fig. 11

Black box testing (BBT) was used to verify the functionality of our desktop application. BBT is a form of testing in which the user has no knowledge of the internals of the software, and they instead observe the output they receive from input they give to the final user interface. Our software has various predetermined use cases, including error messages such as shown in Fig. 11, in response to invalid parameters or displaying GFAP results that we verified as within physiological possibilities using BBT.

4. Originality

From the Team Captains.

The OECT developed by SenseNC for GFAP detection has several novel features. The biosensor's fabrication and biofunctionalization is simple and highly scalable, making our biosensor attractive for large-scale manufacturing. Furthermore, to our knowledge, our biosensor is the first OECT for GFAP detection that utilizes a functionalized gate rather than a functionalized channel. This enables larger surface area for biomarker detection and an enhanced signal response in comparison to functional-channel approaches. Additionally, we developed a streamlined process for creating CNT-composite films with carboxylic acid functional groups. Utilizing these films as gate substrates provides several advantages. First, the surface area of the gate electrode is greatly enhanced due to the irregular surface of the CNT films. Secondly, due to the carboxylic acids present on the CNT films, recognition elements can be directly conjugated to the gate electrode. This eliminates the need for chemical linkers in the bioconjugation process, greatly reducing the number of chemical reagents necessary for functionalization and simplifying the functionalization procedure. Finally, utilizing CNTs as a biosensor substrate and bioconjugation surface greatly enhances the shelf stability of the devices in comparison to traditional self-assembled monolayer based devices. The team independently consulted literature to create a biosensor concept and develop it into a working device. Overall, the biosensor developed by our team is attractive due to its scalability, simplicity, and stability.

Chris Sharkey Kirstie Queener

From the Supervisor.

The 2023 SenseNC Team has made remarkable strides over the past year to design, fabricate, test, and validate the performance of a novel biosensor for glial fibrillary acidic protein (GFAP). The team selected the development of an organic electrochemical transistor (OECT) topology for their biosensor. OECTs have demonstrated unprecedented potential in the field of biosensing due to their unique combination of high sensitivity and label-free operation. The designed OECTs operate based on the modulation of dual-mode charge flux (ionic and electronic) within a conducting polymer channel, resulting in changes in electrical conductance. This mechanism allows for label-free and real-time monitoring of various analytes, making OECTs particularly attractive for biosensing applications with low target concentration and complex matrices. Our students have demonstrated a deep understanding of the principles underlying the engineering of OECTs and their application as biosensors. Their ability to navigate the complexities of device fabrication, materials selection, and experimental methodologies showcases their remarkable growth as junior researchers. Their work highlights the importance of hands-on, experiential learning in fostering true scientific innovation. The SenseNC Team worked independently under the guidance of their graduate student coaches, requiring limited input from faculty supervisors. This included both biosensor development, as well as the development of mobile electronics hardware. Guidance from the Faculty Supervisor was provided on fundamental mechanisms of biosensing, surface chemistry, and limited troubleshooting support on experimental design; while design, fabrication, testing, and data interpretation were the sole responsibility of the SenseNC Team. Logistic and funding support was also provided by the Faculty Supervisor. Nevertheless, additional research and travel funding was attained by the SenseNC Team via competitive University programs (~$5,000). I am immensely proud of the Team's achievements and look forward to seeing how their work continues to evolve and demonstrating their research outcomes at the 2023 SensUs Competition.

Thank you,

Michael Daniele, Ph.D.
Associate Professor & University Faculty Scholar

Department of Electrical & Computer Engineering, NC State University
Joint Department of Biomedical Engineering, UNC-Chapel Hill / NC State University

5. Translation potential

5.1 Business Model Canvas

The business model described here aims to profitably address the need for rapid TBI diagnosis in various settings. The stakeholders and target customers are identified and described. A comprehensive description of the business plan's feasibility and financial viability is provided.

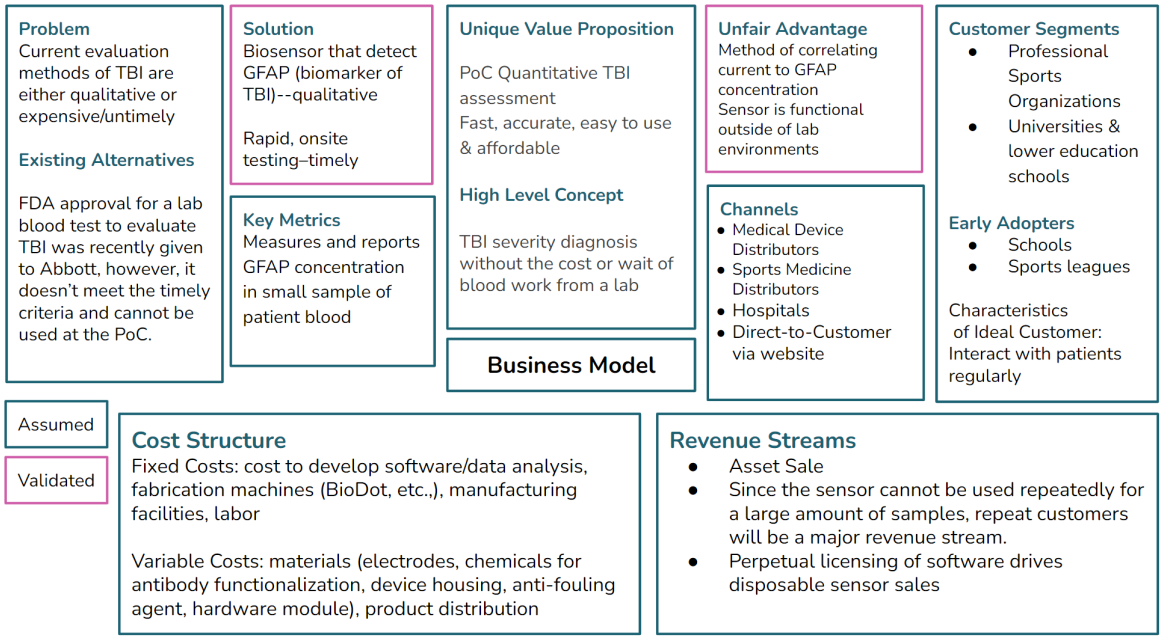


Fig. 12

5.2 Stakeholder Desirability

5.2.1 Customer and Stakeholders

Worldwide, there are approximately 64-74 million cases of TBI each year [6]. According to the Center for Disease Control, in 2020, there were more than 200,000 TBI related hospitalization cases in the United States and more than 60,000 cases resulted in death [7]. Beyond the high incidence of TBI, diagnosis and treatment can be expensive. The estimated direct and indirect (diminished productivity) cost of TBI in the United States amounts to approximately 76.5 billion annually [8]. Diagnosis of TBI is often a time-consuming, expensive process especially when it comes to imaging. CT scans and MRIs are often required to understand the extent of damage especially when the initial assessment indicates the injury is severe. Noncontrast CT scans are typically used to identify intracranial hemorrhages while MRIs are used less often but are particularly useful when the TBI produces non-hemorrhagic injuries [9]. While CT scans and MRIs provide objective data that informs future treatment, they are expensive with head CT scans costing about $200-$2000 and brain MRIs costing about $1600-$8400 [10, 11].

SenseNC proposes a rapid, onsite GFAP biosensor that can identify the concentration of GFAP present in a plasma sample. The results can be viewed on the MATLAB app discussed in the previous section. There are several groups of stakeholders that would benefit from such a device including but not limited to the patient, family members/caregivers, first responders, hospital staff, and school nurses. Our selection criteria are market segments that are sizable, have a specific need for onsite testing, and are affiliated with a medical professional in some way (as opposed to selling directly to customers). Affiliation with a Medical professional is important because the MATLAB interface is better suited for users with a technical background and because medical professionals are more likely to be consistent in their use of the sensor. Our interview with Dr. Perry K. Barnhill revealed that males between 20-40 years and people 70+ years old who are on blood thinners are especially at risk for TBI. We have incorporated this feedback into our market segment focus groups. The same interview also revealed that the learning curve for new medical devices/practices is often steep even for seasoned medical professionals. This feedback led us to believe that it would be better to initially focus on medical professionals rather than regular consumers who would have very little experience using new medical devices. We have identified three scenarios/groups of people that meet our criteria

and benefit from a first-line-of-defense diagnostic biosensor: sports medicine professionals, elderly care staff, and first responders.

There are approximately 1.6-3.8 million cases of sports related TBIs annually in the world and it is estimated that 70% of these cases are in youth aged 10-19 [8, 12]. Athletes are singular in that many do not seek further medical attention after a concussion (often to continue the game) even at risk to their health [8]. An onsite evaluation would prevent having to unnecessarily move the athlete to a clinic and could be conducted on the sidelines. Professional/collegiate athletics often have sports medicine professionals onsite who would be able to operate the biosensor. Current recommendations for sideline evaluation starts with prohibiting the athlete from returning to play until a concrete concussion diagnosis can be made[8, 12]. Concussion diagnosis requires a licensed medical provider to carry out a combination of evaluation protocols including the Sports Concussion Evaluation Tool (SCAT5), Modified Balance Error Scoring System, Post Concussion Symptom Scale, and the Vestibular/Ocular Motor Screening [8, 12]. These tests do not provide conclusive results about the severity of injury and can also be feigned. After this preliminary stage, patients are escalated to CT, PET, and MRI scans if deemed necessary. Our biosensor would eliminate the need for a multiplicity of evaluation protocols.

Another target market is the hospice/elderly care industry. Over half of TBI hospitalizations are related to falls and of fall related TBIs, a majority occur in the 60+ population [13]. Fall-related TBIs in elderly are responsible for 141,998 emergency depart- ment visits, 81,500 hospitalizations, and 14,347 deaths in the United States each year [14]. After a fall, traveling to a hospital or clinic can be physically taxing so patients and their caregivers would benefit from a biosensor that informs them if escalating to the next level of treatment is necessary. Medical professionals at Nursing Homes and Assisted Living Facilities would benefit from having an onsite tool to quickly evaluate the severity of TBI.

Emergency Medical Technicians (EMTs) and Paramedics are stakeholders that would benefit from our biosensor especially in the context of automobile accidents. Data from 1995-2009 suggests that on average, TBIs accidents are responsible for 218,936 emergency department (ED) visits, 56,864 hospitalizations, and 16,402 deaths annually [15]. Having rapid onsite testing could help prevent escalating unnecessarily to sending an ambulance**,** but would also ensure that an appropriate next step is prescribed. Ambulances on average cost $940-1200 so avoiding unnecessary ambulance requests will reduce financial burden on patients while ensuring the resource of an ambulance is saved for more urgent calls [16].

5.2.2 Value Proposition

Abbott Laboratories released a press release on March 7th, 2023 announcing FDA clearance for the i-STAT TBI Plasma test. The press release claims that a reliable result can be provided in 18 minutes and can inform the next step in diagnosis/treatment (decide if CT scan is necessary). The device's test results have a 96.7% sensitivity as well as a 99.4% negative predictive value. Unlike the biosensor SensNC proposes, the Abbott device looks at the presence of two biomarkers–GFAP and Ubiquitin C-terminal Hydrolase L1 (UCH-L1). Another key difference is that Abbott's device was intended for use by lab technicians while SensNCs biosensor does not require a lab environment to conduct tests. Patients that arrive at a hospital provide a blood sample which is taken to the lab where the test is conducted on the Alinity i equipment (also created by Abbott). Abbott has an advantage in this sense because their analysis platform (Alinity i) is already quite established and common in labs [17].

5.3 Business Feasibility

5.3.1 Key Resources

The most vital resources for this business are raw materials, skilled personnel, and facilities for research, development and manufacturing. The raw materials necessary for sensor fabrication are electrodes, chemical reagents (carbon nanotubes, PEDOT:PSS ink, ink stabilizers, anti-GFAP antibodies, conjugation reagents), and PDMS (fluidic cartridge fabrication). Additional raw materials are necessary for enclosure materials, PCB fabrication and packaging. PCB fabrication will be outsourced to large-scale manufacturers until revenue is sufficient to bring this process in-house.

In its current form, the biosensor is functional as a diagnostic instrument. Further optimization and standardization can be accomplished in order to improve sensitivity and batch-to-batch variation. This will require skilled scientists and engineers to enhance the product's technical quality and improve our competitive advantage.

However, due to the functional state of the biosensor, the vast majority of necessary personnel are operators for manufacturing, supply chain management, distribution, and customer support.

Another key aspect of this business's success is regulatory approval and compliance. We intend to target the United States market and will have to seek FDA approval. The process of obtaining FDA approval demands extensive product testing, clinical trials, and regulatory affairs specialists, among other things. However, because a biosensor for GFAP is commercially available and has obtained FDA approval, we will seek premarket approval (also known as the 510(k)) by demonstrating the technological equivalence of our biosensor with commercially available devices [18]. This has the potential to significantly reduce the amount of resources necessary for regulatory approval. Nonetheless, resources will be necessary for quality assurance, postmarket surveillance and compliance with all regulatory standards.

5.3.2 Key Activities

Due to the existing commercially available diagnostic devices for GFAP detection (Abbott), we must use a competitive pricing model. While our device has some key benefits over our competitors (faster time for results, intended for PoC), the only feasible way of initially capturing market share is pricing our device below those that currently exist while offering the equivalent or improved accuracy.

Early company efforts will be devoted to acquiring FDA approval via the 510(k) pathway. This mechanism will significantly reduce the financial burden of seeking full FDA approval. Since we are a brand new company in the diagnostics industry, it is of the utmost importance that we establish our legitimacy by obtaining FDA approval. This will serve as a means of informing all potential customers and users that our products are safe and effective.

Furthermore, our company will file patent applications to protect the specific technique of OECT-based electrochemical detection of GFAP. Patents currently granted to Abbott Laboratories (US10877048B2, US11022617B2) protect the utilization of GFAP assays for TBI evaluation. Thus, our patent efforts will be devoted to protecting specific transduction techniques. These transduction techniques may be generalizable to alternative biomarker detection, and protecting this intellectual property will allow us to develop additional diagnostic tests with the potential profits from our GFAP detection products.

Much of our efforts will also be devoted to establishing partnerships with major sports organizations, as described below.

5.3.3 Key Partners

While FDA approval is being sought, we will actively work to establish partnerships with major sports organizations within the United States. Particular focus will be on the National Collegiate Athletic Association (NCAA) and the National Football League (NFL). We believe that incorporating our diagnostic test into routine player/athlete evaluations will enhance TBI assessment in sports and allow coaches, managers and players to make informed decisions.

In recent years, the NFL has received great amounts of criticism for their mishandling of TBIs. When repeated head injuries occur, individuals are subject to developing chronic traumatic encephalopathy (CTE), which results in a progressive decline of cognitive functioning. In a study conducted by Boston University's CTE Center earlier this year, 92% of ex-NFL players tested positive for CTE, indicating a dire need for more effective assessment of TBI in American football [19]. Furthermore, since 2013, the NFL has paid more than $1 billion USD to former players in lawsuit settlements due to mishandling of TBIs [20]. The NFL has also pledged over $100 million USD to fund research into TBI and CTE protection.

We believe that incorporating our devices into player evaluations and assessments for TBI will not only improve the accuracy of TBI diagnosis, but also alleviate much of the criticism and financial hardships that the NFL has endured due to their mishandling of TBIs. By regularly using our devices, the NFL can show that they take TBI seriously and actively work to protect players. This partnership would be financially beneficial for our company (large order quantities, repeat business, reputable organization for marketing opportunities), the NFL would greatly benefit from our company by demonstrating a commitment to protecting players from TBI.

Partnerships will also be established with materials suppliers and product distributors to ensure consistent product production and delivery to customers. This will be a financially beneficial arrangement for all parties involved, as suppliers will have a consistent, profitable customer and our company will dependably have available inventory.

5.3.4 Sustainability

Our company is committed to sustainable business practices. We use environmentally friendly materials for enclosure fabrication (polylactic acid) and constantly seek new ways to improve manufacturing efficiency in order to reduce waste.

5.4 Financial Viability

5.4.1 Costs Projection

Sensor fabrication currently costs $9.70 USD per device (see below table). It is believed that large-scale manufacturing and procedure optimization can reduce sensor fabrication cost by approximately 25%, resulting in a per-sensor cost of $7.28 USD. Fabrication is largely an automated process and manufacturing one batch of 100 sensors would require roughly three hours of operator time. Assuming that our company pays a competitive operator wage of $20 USD/hr, total cost per 100 sensors would be $787.50 USD, or $7.88 USD per sensor.

Our system's electronics currently cost $437.50 USD per device. Our current prototype utilizes a generalized USB potentiostat (Analog Discovery 2) and a custom amplification board. Shifting electronics development away from general potentiostats and towards specialized hardware will likely reduce cost on a per-device basis. We believe that using our own custom electronics for all of our system's hardware will significantly reduce the cost of our electronics from $437.50 USD per device to approximately $200 USD per device. Many of the features available on our current electronics are unnecessary, and thus can be eliminated to reduce cost. All electronics fabrication will be outsourced to reputable suppliers and the projected costs provided incorporate the costs associated with outsourcing electronics fabrication.

Another significant cost in the business's early life is the fees associated with preparing and submitting documentation for FDA approval/clearance. Based on available data [21], we anticipate the total cost of receiving FDA clearance (testing, preparation, submission fees) to be approximately $500000 USD.

In summary, we project a per-sensor cost of $7.88 USD, and a cost of $200 USD per electronics assembly. Additional costs would be incurred as a result of purchasing clean room facilities, manufacturing equipment, warehouses and shipping/distribution resources (approximately $1 million USD for purchase and $80000 USD for yearly maintenance).

Sensor Materials	**Total Cost (USD)**	**Per Sensor (USD)**	**Electronics Materials**	**Total Cost (USD)**	**Per Device (USD)**
Electrodes	1180.2	7.87	AD2	424.99	424.99
Carbon nanotubes	168	0.13	Amplification Board	37.5	12.5
EDC	178	0.356	Enclosure Materials	19.99	1.999
NHS	135	0.27	**Total**	482.48	439.489
Antibodies	574	0.43			
PEDOT:PSS	288	0.01			
PEGDE	59.8	0.1196			
PDMS	100	0.1			
PEG/BSA	138	0.25			
Cellulose paper	90.38	0.05			

Sensor Materials	**Total Cost (USD)**	**Per Sensor (USD)**	**Electronics Materials**	**Total Cost (USD)**	**Per Device (USD)**
Buffers	19.99	0.1			
Total	2931.37	9.6856			

Table 1

5.4.2 Sales Price

Lab-based biosensors for GFAP detection are commercially available at an estimated retail price of $16 USD per sensor with an electronics cost of approximately $10000 USD [22]. Despite our competitive advantage of being a PoC device, it is imperative that our sensor's pricing is competitive with such products in order to capture any market share. Therefore we intend to sell each biosensor at a price of $15 USD, and our electronics will retail for $2000 USD.

5.4.3 Market Analysis

There is currently no commercially available device for point-of-care quantification of GFAP for TBI diagnosis. Lab-based devices are available (Abbott Laboratories i-STAT TBI Plasma Test), but such products are inaccessible for rapid evaluation of TBI in athletics and accidents at the point of care.

We intend to initially target the professional sports market, with a particular focus on the National Football League. We believe that a partnership would be mutually beneficial. There are 32 teams in the NFL and 1696 active players [23]. We predict that our device would be incorporated into player evaluation on a biweekly basis. We expect that each NFL team would own at least three of our electronics devices for efficiency and convenience of testing, indicating one-time sale of at least 96 devices. . Assuming that a diagnostic test would be used on each NFL player 26 times per year, in addition to one diagnostic test per concussion event (approximately 150 concussions per year), 44246 sensors would be used by the NFL per year.

We also intend to develop a partnership with the NCAA in order to incorporate our technology into annual physicals of collegiate athletes. There are approximately 522000 athletes in the NCAA. Assuming that we can establish a partnership with NCAA American Football for yearly player GFAP testing (approximately 15000 players) and an additional 10% of NCAA athletes, 65700 diagnostic tests would be used by the NCAA per year.

Targeting the two markets of collegiate athletics and the National Football League and pursuing strategic partnerships would result in 109946 sensors being used annually. Assuming that sensors are sold for the stated retail price of $16 USD and electronics are sold for the stated retail price of $2000 USD, the annual revenue from these two organizations would be sufficient to sustain the business, establish market share, and develop a presence in markets beyond athletics, such as emergency medical services.

5.4.4 Revenue Streams and Business Strategy

Revenues will be generated by one-time sale of electronics and repeated sales of biosensors. Using the market analysis above, expected annual revenue from sensor sales alone would be approximately $1.76 million USD. One-time sale of electronics would result in $192000 USD of revenue from the NFL alone. The anticipated cost of such revenue (109946 sensors, 96 electronics devices) would be $885575 USD yearly. The net profit of this business plan would be approximately $900000 USD annually. Assuming that virtually all profits would initially be used to cover wages (approximate $400000 USD), debts accrued from FDA approval ($500000 USD), and facility and equipment purchases ($1 million USD), the company would reach its breakeven point in approximately three years.

6. Team and support

6.1 Contributions of the team members

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.

Angelica Aroche, Kaila Peterson, Jack Twiddy, and Junhyeong Wang:
Angelica Aroche, Kaila Peterson, Jack Twiddy, and Junhyeong Wang 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.

Abisha Fenn
Abisha Fenn is a member of the electrical, business, and social teams. Abisha coordinated all the undergraduates for funding purposes, and also heavily contributed our electrical hardware as well as business slides and presentations.

Misk Hussain
Misk Hussain is a member of the electrical, chemical, and social teams. Misk was involved with the researching of our protocols and the choosing of our electrical hardware, as well as documentation.

Jacob Linnabary
Jacob Linnabary is a member of the electrical and social teams. Jacob was heavily involved in our social media posting and helped with the research of our electrical hardware.

Lina Acosta-Perez
Lina Acosta-Perez is a member of the chemical and social teams. Lina was very crucial with the finalization of our functionalization protocol and manufacturing of our functionalized electrodes.

Kirstie Queener
Kirstie Queener is a team captain and a member of the chemical and social teams. Kirstie played a huge rule in the coordination of the team, as well as headed the chemistry for the project.

Christopher Sharkey
Christopher Sharkey is a team captain and a member of the electrical, chemical, and business teams. Christopher was heavily involved in both the electrical and chemical parts of the project, while also taking a big role in our business side and interacting with partners.

Liam Wyman
Liam Wyman is a member of the electrical, and business team. Liam was involved with the research and design of the sensor, as well as business slides and presentations.

Max Yates
Max Yates is a member of the electrical, and business teams. Max was responsible for the sensor documentation and work. He heavily contributed to the design and testing of electronic hardware.

6.2 People who have given support

Mahshid Hosseini
Mahshid served as the data official for the DTE. She also was consulted about electrode cleaning techniques.

Gabby Rusch
Gabby served as the sample official for the DTE.

Halston Deal
Halston was consulted about PDMS and developing PDMS molds with a microfluidics 3D printer.

6.3 Sponsors and partners

PalmSens
PalmSens was our partner for Partner Sessions, but also met with our team outside of the Partner Sessions. PalmSens provided us with feedback about our chemical and electrical components, as well as answering specific electrochemical questions we had.

7. Final Remark

SenseNC's team members would like to extend their gratitude to our coaches, mentors, and industry partners that helped us throughout this process. We are also grateful for the opportunity to participate in the SensUs Student Competition and everything we have learned by participating. Our work was also made possible by North Carolina State University's Office of Undergraduate Research, the Engineer Your Experience program, and the National Science Foundation.

We look forward to continuing our work on OECT-based biomarker detection.

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