Review—Opportunities for Rapid, Sensitive Detection of Troponin and Cerebral Spinal Fluid Using Semiconductor Sensors

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There are opportunities for development of modularized, inexpensive protein biomarker sensors in clinical applications. In this review we focus on two of these, namely early diagnosis of acute myocardial infarction (AMI) and detection of cerebral spinal fluid (CSF). Evaluation of patients with acute chest pain is challenging due to the heterogeneity of the underlying conditions, leading to patients with AMI being mistakenly sent home from emergency rooms or those at low risk for an adverse cardiac event being unnecessarily admitted without precise cardiac biomarker testing. Cardiac troponin I (cTnI) in cardiac muscle tissue is a standard clinical biomarker for AMI, as its concentration rises quickly in the blood during release from myocardial cells following cell death. The time-dependence of the cTnI concentration is the basis of antigen-antibody methodologies such as radioimmunoassay and enzyme-linked immunosorbent assay (ELISA). These methods are time consuming, leading to delays in diagnosis and higher costs. The challenge is to develop a real-time, accurate, low-cost point-of-care heart attack sensor. The coefficient of variation must be precise, within the parameters established by the American College of Cardiology. Similarly, leakage of cerebrospinal fluid (CSF) is a critical condition with a high risk of meningitis and potential mortality. The primary methods of detection for the biomarker β2-Transfferin (B2T) are immunofixation electrophoresis (IFE) and ELISA. Consistent IFE results down to 2 μg/mL can be obtained in patient samples, but requires a minimum 2.5-hour testing period, which is not expedient for real time feedback during surgery in or around the central nervous system. Additionally, to achieve good sensitivity and handle the inherently low concentration of B2T in CSF, lab procedures require samples to be concentrated or run in duplicate to ensure accurate detection. Real time turnaround is on the order of days. To alleviate the slow turn-around times, there is strong interest in electronic detection methods for proteins using biologically functionalized transistors, which provide an electronic readout and are readily integrated with wireless data transmission.
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The compound annual growth rate of biosensor sales from 2019–2025 is predicted to be 8%, growing from the 2018 market size of $US18.6B.[1] There are many companies developing wireless sensors for continuous monitoring of patient vital signs to take advantage of the higher data transfer rates in the new 5G mobile networks. A basic biosensor consists of the recognition layer (often an enzyme or antibody), the sensor element which transduces a signal of interest, amplification of this signal, signal processing and a display for data readout. Semiconductor-based sensors offer numerous advantages, including high sensitivity, easy scale-up, low cost and rapid response. We have identified two specific clinical applications which would benefit from developing new biosensors, namely early confirmation of heart attacks and detection of cerebral spinal fluid leaks.

Cardiovascular disease (CVD) is the cause of more deaths than cancer, chronic lower respiratory diseases, and accidents combined.[2–15] Roughly 84 million people in the US suffer from cardiovascular disease, causing about 2,200 deaths per day, averaging one death every 40 seconds.[2] CVD is an economic burden for the healthcare systems in industrialized countries. The global cost of CVD is estimated as $108 billon per annum, with $65 billon attributed to direct and $43 billon to indirect costs.[11] The US is the biggest contributor to the global CVD costs and is responsible for 28.4% of total global spending, compared to 6.8% for Europe.[11] Early diagnosis (ED) of AMI is essential for patients with evolving MI or at high cardiac risk to be identified for quickly receiving the appropriate level of healthcare. The initial evaluation of patients with acute chest pain represents a challenge of heterogeneity of underlying conditions, which may miss MI inpatients mistakenly sent home from the emergency department or result in hospital overcrowding by the admittance of patients with low risk for an adverse cardiac event.[2,9]

Patients with chest pain typically account for ∼10% of all ED admissions, but only around 5–10% of these have a confirmed diagnosis of MI at discharge.[9] For the remainder, symptoms are often not life-threatening conditions.[12–16] The key objectives of early assessment is to rapidly identify low risk patients for safe discharge from the ED and patients with evolving MI or at high cardiac risk to receive adequate level of healthcare.

Cerebrospinal fluid (CSF) is a physiologically critical extracellular liquid secreted from the choroid plexus in the cerebral ventricles.[17–21] CSF covers the brain and spinal cord, held in the central nervous system by the meninges. In addition to acting as a physiological buffer solution, providing nutritional and waste transport, it also helps maintain intracranial pressure and acts as a physical shock absorber, cushioning to the brain in case of sudden movement or force. Much like blood and other body fluids, CSF is constantly replenished. Despite a standing volume of 125 to 150mL, approximately 600 mL of CSF is produced daily, refreshing the entire standing volume every 6 hours. A CSF leak is a serious complication that can result from traumatic, iatrogenic, or spontaneous connection between the intradural and extradural spaces.[22–26] The primary concern related to CSF leaks is meningitis and intracranial infection, which left untreated can be fatal. Common symptoms include headache, nasal drainage, ear drainage, fever, and tinnitus. While imaging studies can often elucidate the site of a leak, the gold standard for detection of CSF is performing an assay for the protein Beta 2 Transferrin (B2T) in nasal secretions or other drainage.[27–30] While this test results in 99% sensitivity and 97% specificity,[17–27] it generally relies on off-site laboratories with turnaround time on the order of a week for a result. This results in a delay in definitive diagnosis and treatment for patients, especially in the case of spontaneous CSF leaks.

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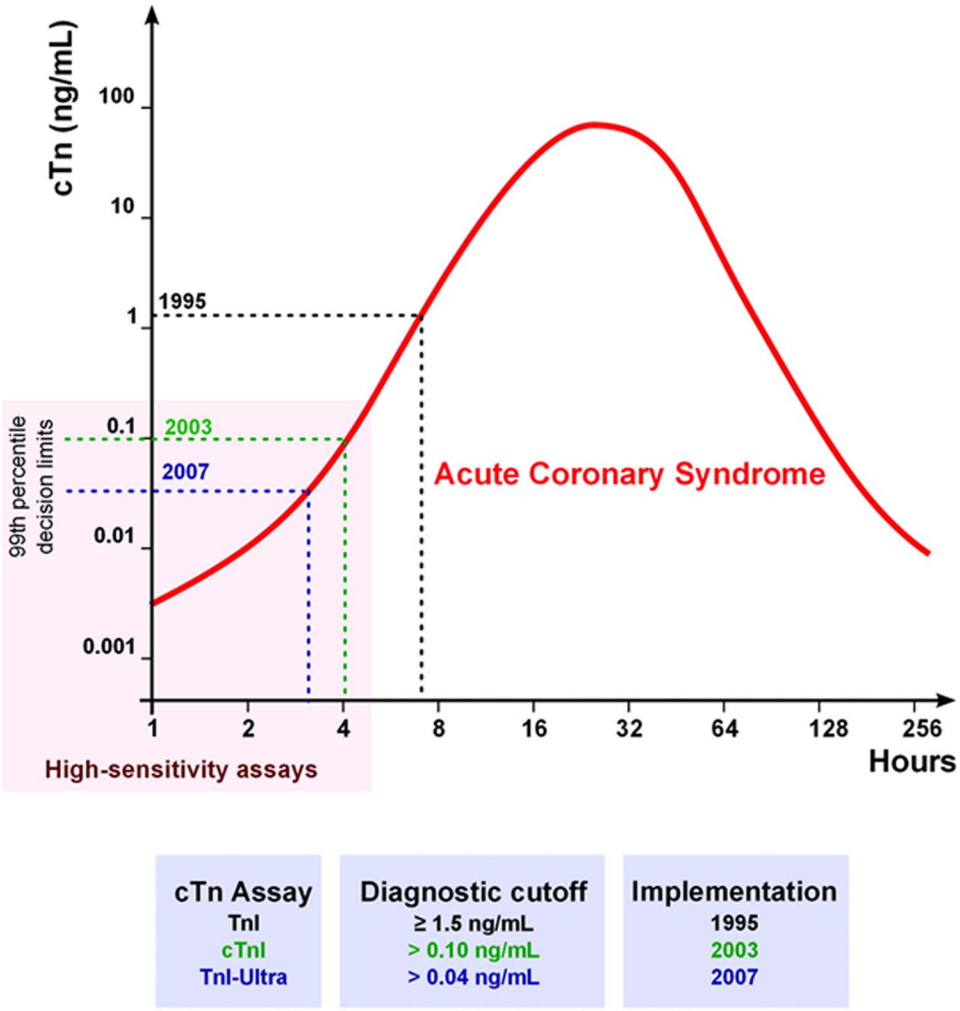

Figure 1. Advancement of the cTnI assays and their diagnostic cutoffs. A hypothetical case of acute coronary syndrome represents with the earliest times of potential diagnostic cutoffs of more sensitive cTn assays. The years correspond to the implementation of the respective assays in the US market.[13]

In this article, we review recent progress in developing point-of-care, handheld sensors for biomarkers of AMI and CSF leaks and identify where these can provide the maximum benefit in a clinical setting and the challenges still remaining in ensuring these sensors are robust and useful.

Cardiac Troponin Testing

Currently, cardiac troponin (cTn) assays for detection of cardiac injury has become the standard of care.[9,15] Cardiac troponin is elevated if serum/plasma concentration exceeds the 99th percentile of a normal reference population. cTn are proteins mainly found in the sarcoplasmic reticulum of a cardiac myocyte, with small amounts also existing in the cytoplasm.[3,4] The cTn complex consists of three subunits - an inhibitory component (cTnI), a tropomyosin binding component (cTnT), and a calcium binding component (cTnC). cTnI and cTnT are specific for cardiac myocytes, and hence, used as markers for cardiac injury detection.[4,7,8,15] cTn are released within two hours of the onset of cardiac injury symptom development, peak at 12 hours, and remain elevated for 5–14 days, with a bell shape response, illustrated in Figure 1.[13] The sensitivity of these cTn assays has progressively increased over the years,[3,4,13] allowing faster diagnosis. The first generation of cTn assays for the diagnostic cutoff of acute coronary syndrome (ACS) was set as 1.5 ng/mL in 1995. These cutoffs have since decreased to 0.1 ng/mL with more contemporary assays and have limits of quantification down to as low as 0.04 ng/mL with high sensitivity-cTn (hs-cTn) assays.[3,4,13] In addition, hs-cTn assays also have a lower limit of quantitation defined as 10% of coefficient of variation (CV), to provide higher precision of cTn determination.[13] Figure 2 shows an illustration of an algorithm based on the European Society of Cardiology for rapidly ruling-out of MI patients using hs-cTn arrays.[3] The effectiveness of this approach is obviously dependent on the sensitivity and speed of the assay used to determine the cTn concentration in the patient. An area of opportunity is to develop hand-held hs-cTn assays with fast response times.

Both contemporary and hs-cTn assays are based on antigen-antibody based interactions using techniques such as radioimmunoassay and enzyme-linked immunosorbent assay (ELISA). These methods are time consuming. Thesemultiple-step processes require expert personnel to perform tests which canlead to delays in diagnosis if staff are unavailable and higher costs. For example, the number of blood samples going through the pathology laboratory in our local Shands hospital at the University of Florida is 8 million samples annually. Even with the implementation of an expensive automated systems, the turn-around time of cTn testing is around 30–90 mins. Emerging data indicates point-of-care (POC) testing in CVD could facilitate more rapid test results, but POC is not commonly employed due to lower sensitivities and higher limit of quantitation.[31] As POC testing continues to evolve, precision is expected to improve. With a continual decrease in test turn-around time, triage time, and associated health care costs, these POC tests may advance emergency cardiac care.

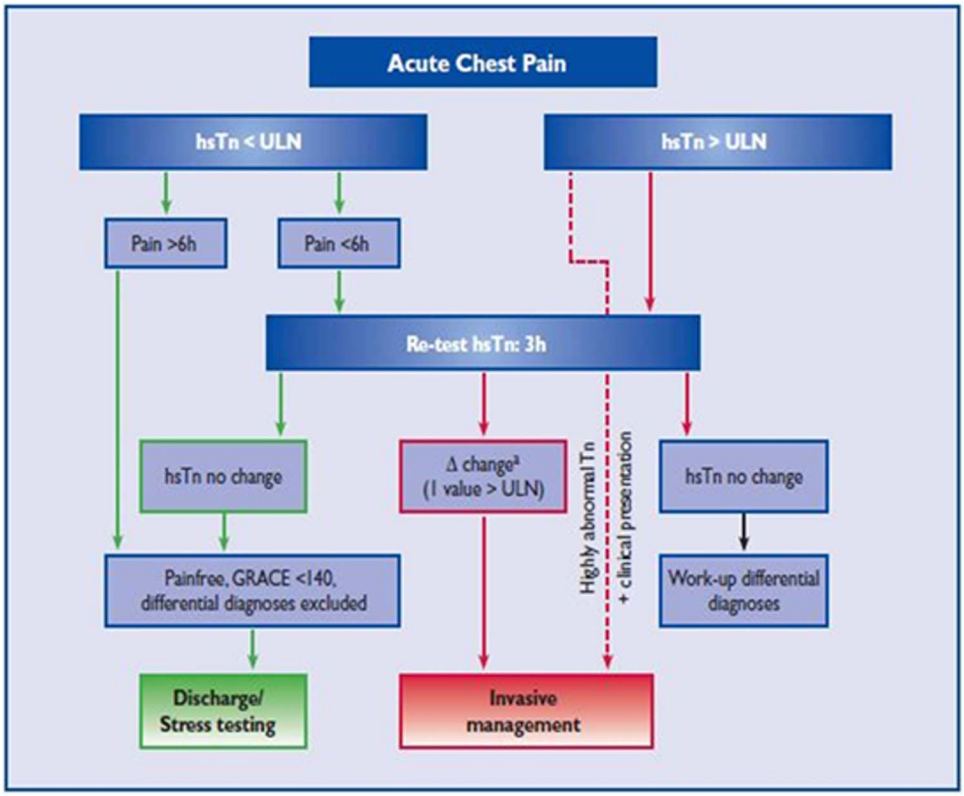

Figure 2. Algorithm based on the European Society of Cardiology guidelines for rapidly ruling-out of MI patients using hs-cTn arrays[3] (copyright Springer, reprinted with permission).

The challenge is to develop a real-time, accurate, handheld, wireless capable and low cost cTn sensor with similar sensitivity to hospital laboratory assays. The advantages of hs-troponin assays are improved precision for the detection of "rate of rise" of troponin earlier (at much lower concentrations in the blood) than were previously possible. This leads to a shorter protocol for the evaluation of chest pain. A guideline issued in 2007 by the National Academy of Clinical Biochemistry states "in the presence of a clinical history suggestive of ACS, the following is considered indicative of myocardial necrosis consistent with myocardial infarction:[5,7] maximal concentration of cTn exceeding the 99th percentile of values (with optimal precision defined by total CV [coefficient of variation] 10%) for a reference control group on at least one occasion during the first 24 hours after the clinical event." This rule provides the framework for deciding the decision limit or a "positive" troponin result. Based on the 99th percentile rule, it is possible to identify patients with ACS earlier with hs-cTn assays, enabling earlier coronary intervention, as illustrated in Figure 1.[13] The advantage of increasing analytic sensitivity may have a trade-off of reducing specificity to present an additional diagnostic challenge for clinicians for the current hs-cTnI technology. With the capability of a lower detection limit of 0.01 ng/mL and fast testing turn-around time of the disposable sensor approach, it would allow monitoring the real time dynamic cTnI concentration change to increase the specificity.

For high sensitivity troponin detection this means it must be possible to achieve

1) Limit of Quantitation (10% CV) must be lower than the 99th percentile.
2) at least 50% of samples from healthy individuals must have detectable levels i.e., must exceed the LOD
3) Must detect sex-specific 99th percentile upper reference limits for normal healthy individuals.

Table I shows a comparison of assays currently in use. Note that some use detection of the troponin-T biomarker and some use troponin-I. There are also differences between male and female patients in terms of the 99th percentile values. It should also be noted that there are a number of analytic interferences with troponin measurements, including hemolysis, icterus, lipemia, heterophilic antibodies, autoantibodies, anticoagulants and conditions of sample storage.

The limit of quantification (LoQ) is the lowest concentration at which the analyte can be reliably detected AND at which some predefined goals for bias and imprecision are met. The precision and coefficient of variation (% CV) are important in this regard. A precise test gives the same or near-identical test result every time the same specimen is assayed. This is quantitated by the % CV - the ratio of the standard deviation (SD) to the mean (SD ÷ Mean) × 100. The Limit of Blank (LoB) and Limit of Detection (LOD) are defined as follows: LOB - if a readout is < LOB, you are 95% certain that there is no analyte present in the specimen. LOD - if a readout is > LOD, you are 95% certain that an analyte is indeed present in the specimen. The

Table I. Comparison of troponin assays currently in use in clinical settings. The high sensitivity assays have different values for the 99th percentile for Males(M) versus Females (F).

Company/Platform/Assay	88th Percentile	10% CV
Contemporary Assays (ng/mL)		
Beckman Coulter DxI	0.03	0.04
Roche 4th Gen cTnT	0.01	0.03
POC Assays (ng/mL)		
Abbott i-STAT	0.08	0.10
hs-Assays(ng/L)		
Abbott ARCHITECT hs-cTnI	34/16 (M/F)	3
Beckman Coulter Access hs-cTnI	52/23 (M/F)	8
Roche E170 hs-cTnT	20/13 (M/F)	13

Table II. Comparison of troponin assays. All measurements are in ng/L.

Commercial Assay	LoB	LoQ	99th %	%CV 99th %	10% CV	Interferences
Abbott Architect STAT hs-cTnI	0.7–1.3	3	23	4.0	4.7	
Beckman Coulter AccuTnI+3	<10		56	10	N/A	
Beckman Coulter hs-cTnI	<0.3	8	21	3.0	5	No interferences with hemolysis, bilirubin, lipids, heparin or fibrinogen
Roche hs-TnT	N/A	13	15	14	13	
Abbott i-STAT	20	N/A	39	80	16.5	

Functional Sensitivity or Limit of Quantification of cardiac troponin is the lowest concentration that gives a CV of $\leq$ 10%. Table II gives a comparison of all these parameters for current clinical assays in use at Shands.

Cerebrospinal Fluid Testing

The β-2 conformation of transferrin is carbohydrate-free and found almost exclusively in CSF (it is also found in perilymph from the inner ear[20,21]), but not mucous, blood, tears, or serous fluid. Once fluid drainage is confirmed to be CSF, imaging is performed to confirm the specific area of leakage. High-Resolution Computed Tomographic (HRCT) scans are most often used for identification of skull base CSF leaks as they can provide sub-millimeter sections in the coronal and axial planes.[29,30] Surgery is performed to seal the leak, ultimately preventing further drainage and infection.[39–43]

Computed tomography (CT) and magnetic resonance imaging (MRI) can play a secondary role in localizing a leak, but often will fail to demonstrate a defect or identify the specific site of CSF leak.[32–43] More invasive testing, such as intrathecal injection of radiopaque or radioactive compounds can assist with making CT and MRI diagnosis more accurate; however, it can only confirm the diagnosis when leakage occurs during the time of examination and may fail in cases of intermittent CSF leakage.[20–28] Intrathecal administration of fluorescein with surgical exploration may reveal the specific site of leak, but results in the risks associated with anesthesia, and costs associated with mobilization of the surgical suite and the required post-operative care.

Currently, there are two primary methods of CSF detection in fluids: immunofixation electrophoresis (IFE) and enzyme-linked immunosorbent assay (ELISA).[24–28] IFE relies upon the separations of proteins by their molecular weight, with the β-2 conformation of the transferrin protein traversing farther along an acidic gel than the β-1 conformation. Papadea et al.[27] demonstrated consistent IFE results down to 2 μg/mL in patient samples, but this result required a 2.5-hour testing period which is not expedient enough for real time feedback.[37,38] Additionally, to achieve good sensitivity and handle the inherently low concentration of B2T in mixed fluids, laboratory procedures have required samples to be concentrated by as much as 10-fold or the sample to be run in duplicate to ensure accurate detection.[29–34] The practical reality is that even though both tests result in 2.5 to 3.5 hours, testing is performed at a small number of sites throughout the country, creating an actual result time of days to a week.

Functionalized Semiconductor Sensors

To address the limitations of turnaround time and limited detection thresholds, there has been strong interest in electronic detection methods for proteins, viruses, or small molecules using biologically functionalized field effect transistors (FETs). Much of this work revolves around bio-functionalized AlGaN/GaN high electron mobility transistors (HEMTs), due to the excellent sensing characteristics from the high-density electron channel located near surface (~25 nm). This type of sensor platform has been used extensively for a wide variety of bio-sensing applications,[44–53] but suffers from a number of disadvantages, chiefly the high cost of HEMT devices. For protein sensing, one of the major concerns can be the high ionicity of the test solution (secretions/blood/serum). The high ionicity can cause charge screening effects where a critical detection factor, the Debye screening length, is actually shorter than that of the protein. To circumvent this, a double pulse measurement has been developed which provokes a spring-like response of the antibody-protein complex that can be then sensed regardless of the high ionicity of the solutions.[54–61] A schematic of the sensor approach is shown in Figure 3, using a functionalized glass slide that is externally connected to a HEMT.[44,62,63] This approach makes the device reusable as a testing strip is replaced each time. A significant limitation of this method is still the high sensor cost.

Chemical analysis of the discharge fluid is a noninvasive method, as B2T is found almost exclusively in CSF. Surprisingly, this assay only provides a binary result, is slow, and does not provide a quantitative result. For example, fluid detection of B2T by agarose gel electrophoresis on Beckman Paragon equipment, followed by pressure transfer to nitrocellulose and incubation with enzyme-labeled anti-transferrin antibody and substrate took 3.5 h with sensitivity 1 $\mu g.ml^{-1}$ [21]. This presents an opportunity: a point of care test with a sensitive and specific result available in minutes that also provides a quantitative result.

Recent Progress

The advancement of clinical biosensors has been hindered by the charge screening effect, restricting the use of FET based biosensors to low electrolyte test samples, and consequently affecting the binding kinetics and integrity of biological samples.[54–56] While methods have been proposed to overcome this screening effect, which would allow for screening of whole blood and other unprocessed samples, these require elaborate and complicated pretreatment methods, such as repeated washing and enrichment, dilution, and desalting.[54] This pretreatment adds to the complexity of the biosensing system without ensuring preservation of the physiological environment and may

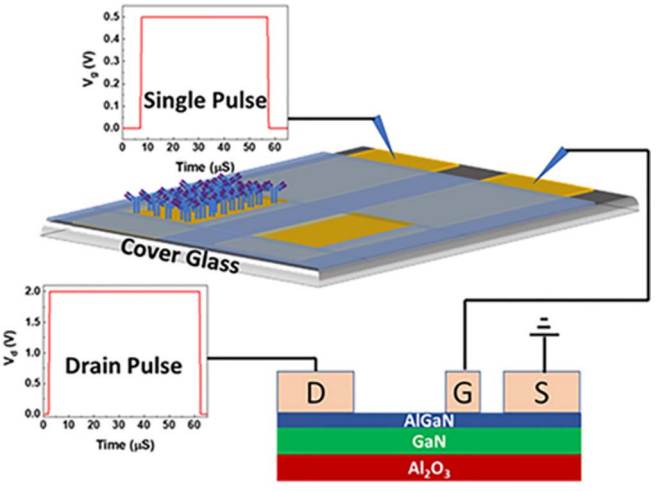

Figure 3. Schematic of an externalized sensor that removes need for cleaning and any mishandling of the expensive HEMT. The replaceable cover glass is cost-effective and the capacitance of the sensor may be increased by increasing the surface area of the cover glass electrodes.

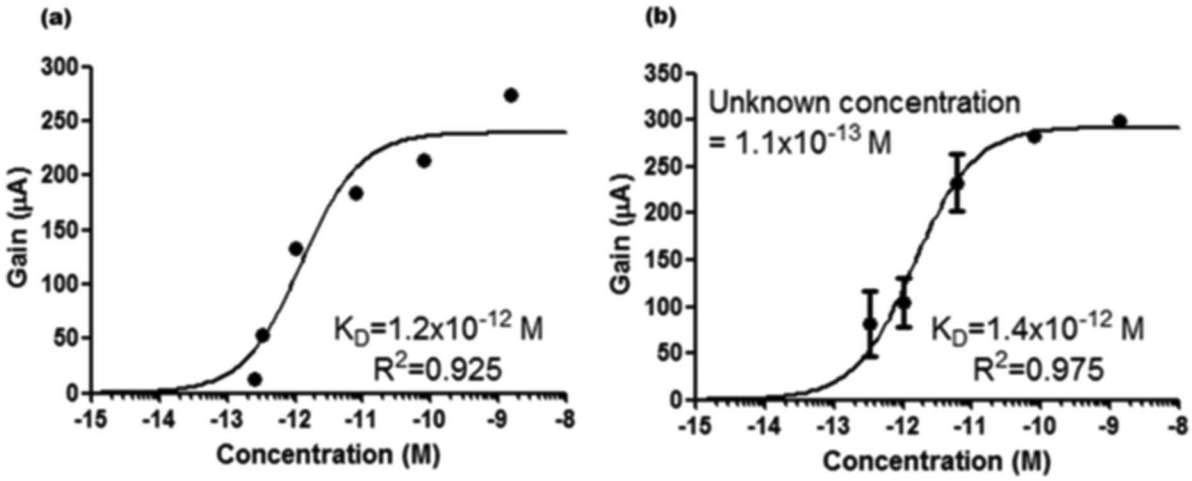

Figure 4. (a) Binding site model for the detection of purified Troponin I samples. (b) Binding site model for the detection of Troponin I in human serum samples (after Sarangadharan et al.[56]).

compromise reliable biological signal acquisition. As a result, the current iterations of these sensing methodologies do not provide sufficient advantage to obviate the conventional laboratory-based spectroscopic techniques.

The group of Y. L. Wang[54–61] developed an electrical double layer (EDL) gated FET biosensor capable of the direct detection of target analytes in physiological buffer solutions without extensive pretreatment or washing. An example of the detection of troponin I in PBS solution with 4% BSA is shown in Figure 4a, along with fitting to a simple one-site bonding model, while Figure 4b shows the data for detection of troponin in a human serum sample.[56]

To further simply the assay, it is necessary to separate the blood cells from plasma. It would be requirement that similar troponin concentration can be detected in both blood plasma and whole blood with a microfluidic channel based red and white blood cell filter integrated with the sensor chip to eliminate the conventional process of fractionating the whole blood sample to obtain plasma. Blood consists of plasma, red blood cells (RBCs), leukocytes and platelets. Plasma and cells constitute the two main blood components each with ∼60% and 40% volume fractions respectively. Fractionating the various target components from blood has been a challenging problem both from a medical and engineering perspective. Typically, plasma is extracted from blood in laboratories and clinics by centrifugation with conventional bench-top centrifuges which are known to be expensive, time consuming and labor intensive. Microfluidics based blood component separations have recently been employed to resolve the issue of membrane clogging and compromise separation efficiency, especially for the application of small sample volumes.[64–72] A schematic of an integrated microfluidic device is shown in Figure 5, while Figure 6 shows an entire system for clinical use, in which the blood can be analyzed and the result shown on a hand-held instrument or a laptop.[57]

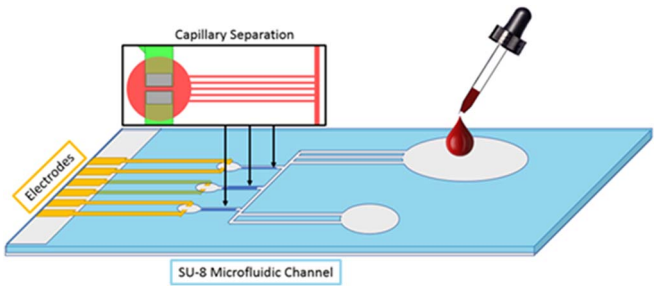

Figure 5. Proposed multiple microfluidic channel device fabricated on the plastic strips with optimized electrode configuration.

A key part of this development is to simplify the instrumentation, e.g., replacement of the expensive HEMT device with an inexpensive Si transistor and use of a disposable plastic, glass, or paper cartridge strip onto which the bodily fluid sample is placed. This cartridge is then inserted into a hand-held instrument containing the associated electronics for readout, data recording and wireless transmission, if needed. The cartridge is electrically connected to a printed circuit board test specific module within the permanent hand-held device. Figure 7 shows the current state-of-the-art of our integrated circuit board for signal processing and display. The sensing methodology is based on an extended gate EDL-FET biosensor that can offer very high sensitivity, a wide dynamic range, and high selectivity to target analyte. By adopting this approach, the cost of the sensing system is reduced, the sensor is capable of detecting target proteins over a much wider range of concentrations without dilution or sample pretreatment and there is a direct readout of protein concentration, obviating the need for user interpretation.

Our preliminary data shows this arrangement works for saline solutions containing known quantities of B2T, simulating CSF, at clinically relevant concentrations.[44] Time dependent detection of CSF dilutions from 0.1 ng/mL to 100 ng/mL are shown in Figure 8 (top). The curvature of the responses results from the dynamic drain current response due to the double spring-like response of the perturbed antibody protein complex. The average gains across five separate sensors are shown at the bottom of Figure 8. The concentration is of CSF as a whole, while we are truly only targeting the β2T, which is a minor component of the proteins of CSF. Normal CSF contains < 1 g of all types of present proteins per 1 L of CSF; thus the actual tested concentration for β2T is at least 1000X fold more diluted than the stated CSF.[44]

This data can be fit to a Langmuir Extended Isotherm model for small molecules to relate the gain of the sensor to target concentration[73] and while this provides an adequate fit (Figure 9), a more accurate approach involves relating solution concentration of the protein to the bound fraction of sensor surface.[74,75] This Stankowski model also provides an accurate predicted maximum current and information on the binding kinetics of the protein to the sensor surface.[44] The detection limit of 0.1 ng/mL is well below current clinical detection levels (1300 ng/mL is the level indicating the presence of a CSF leak, whereas concentrations of <700 ng/mL indicate the absence of a leak).

This work is progress toward realization of a portable system capable of performing the diagnostic assay simply and rapidly, with a user able to screen for CSF presence from a single drop of secretion, in 5 minutes or less. This approach is attractive for inexpensive cartridge-type sensors for the detection of B2 directly from different body fluids.

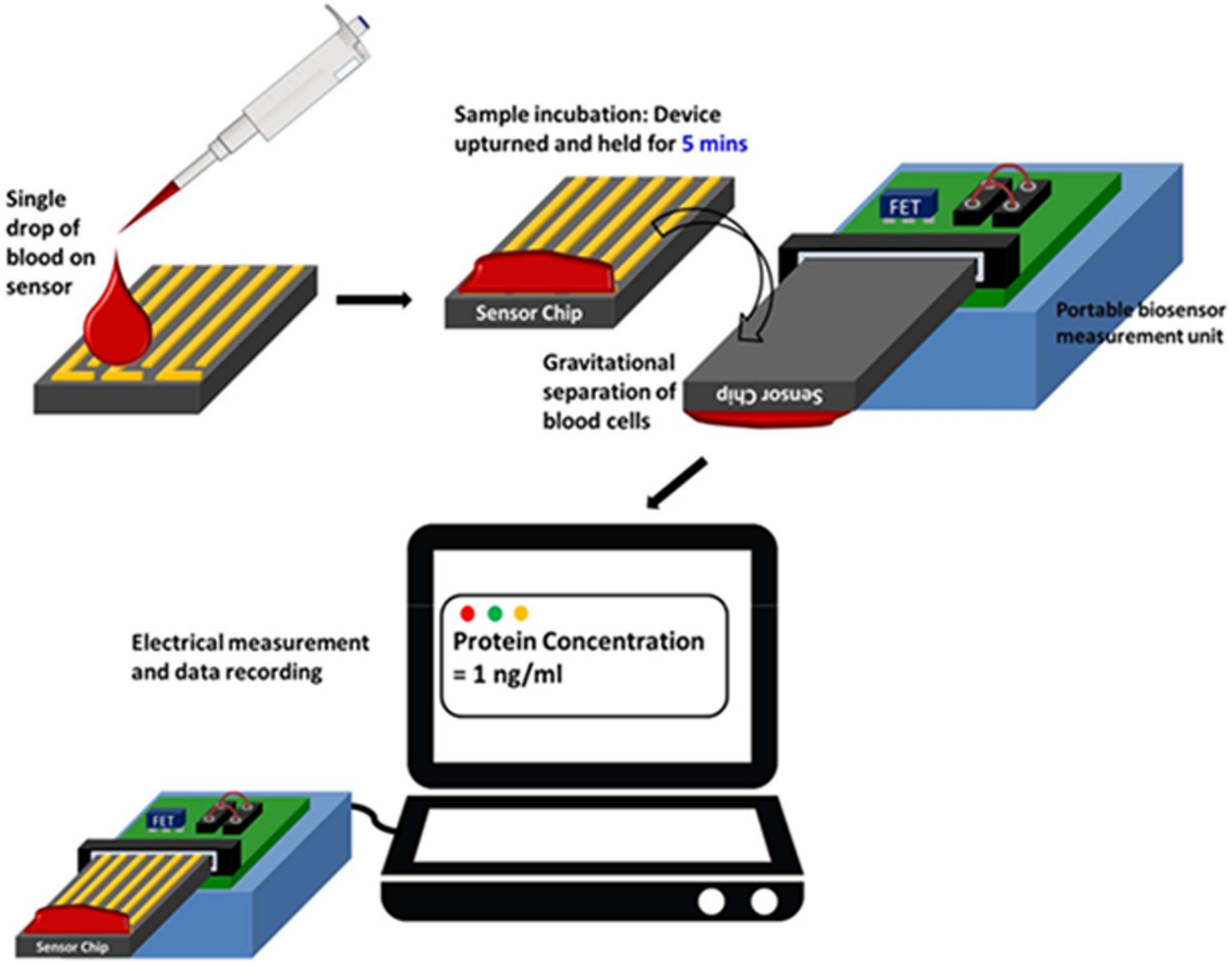

Figure 6. Assay for cTnI detection using portable FET biosensor system. The blood sample is placed on the sensor chip, followed by gravitational separation of blood cells and then carry out electrical measurement using the software installed in the personal computer and obtain results instantaneously.[57] (copyright American Chemical Society, reprinted with permission).

Future Research

There are issues that still need additional study. In terms of the troponin detection, we need to understand the ability of our sensor to accurately detect proteins in whole blood, which is relevant to mapping the detection technology to other proteins. This is essential as a POC sensor cannot be dependent on significant pretreatments such as desalting, dilution, centrifugation, or the other myriad processes used by central laboratories. A significant challenge is that the red blood cells in whole blood will often precipitate out onto the sensor surface, blocking part of its response area. When untreated blood is dropped on our sensor surface, it starts to coagulate in 4−5 min. Delineating how these samples must be handled, and whether or not

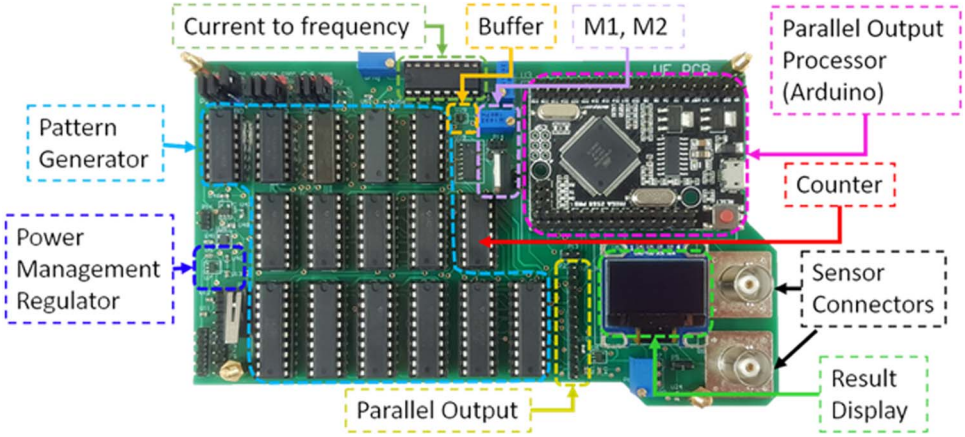

Figure 7. Photograph of a prototype design, including readouts, microcontroller, signal processing units, and display. BNC connectors are used for the current design, and they will be replaced with strip clip connectors once the configuration of the sensor chip is optimized.

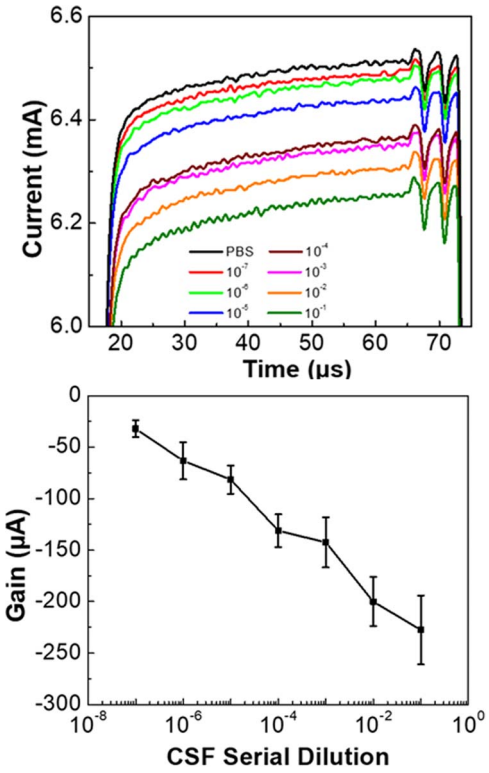

Figure 8. (top) Current response for beta 2 transferrin of different concentrations in PBS solution applied to a glass slide and (bottom) signal amplification as a function of beta 2 transferrin concentration. This determines the dynamic operating range of the sensor.

anticoagulants or chelating agents must be added to the sensor is essential to standardizing target detection.

In terms of CSF sensing, while we have demonstrated the sensors ability to detect pooled B2T in saline buffer, we first need to confirm that our sensor will maintain its characteristics with clinical, non-purified, samples of CSF. This understanding relies on developing a protocol for reproducible application of CSF and other fluids to the sensors with high reliability by a wide range of users and understanding the effect that ancillary proteins and solutes can have on the ability of the sensor to accurately measure presence and concentration of the target. This is important as real-life samples will be admixed with mucous, bacteria, blood, serous fluid and a host of other confounders. In fact, it would also be useful to understand the how concentrations

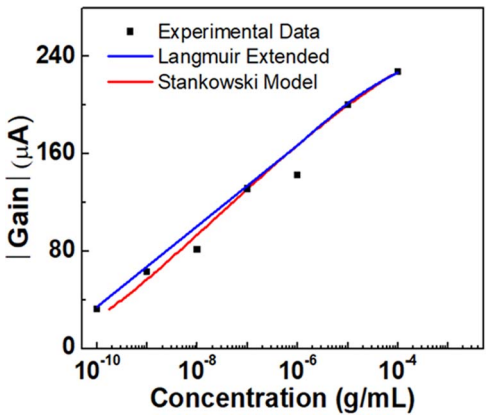

Figure 9. Experimental data and fits to Langmuir-Extended and Stankowski model of absolute sensor gain as a function of CSF test solution concentration.[44]

of B2T differ in target samples (e.g nasal secretions) from that of CSF obtained from lumbar drains. It is also possible that other body fluids may have B2T, but in concentrations that previously have been unquantifiable.

The last stage is the further development of a POC device. The initial iteration of the sensing system required the use of a large benchtop waveform generator and oscilloscope to manage the circuits, sensor, and output. We have miniaturized the electronics, placing the waveform generator and oscilloscope on the chip board itself and adding a readout display. With the understanding that this device is to handle mixed and unprocessed fluids, we are working on the second iteration of the sensor, which has been designed to incorporate micropost and capillary action filtration and will be made of plastic in lieu of glass. Further refinement of this sensor also includes the distances between the sensory electrodes that measure the current across the medium, as well as the area of anti-B2T coating on the electrode tips. We anticipate that different proteins may have different dynamic ranges based on both of these factors and being able to make this to the widest range of proteins would reduce the overall per use cost of a sensor. Besides sensing sensitivity, durability and cost of the sensor chip are two of the most important requirements for protein biomarker test strips.

Conclusions

We have reviewed two clinical opportunities where highly sensitive semiconductor-based sensors may have advantages. The first is detection of cardiac troponin (cTn) I and T proteins released from myocardial cells following necrosis. The ability to make a sensitive and reproducible measure of cTn concentration in blood following ischemia/chest pain can enable diagnosis of whether myocardial infarction (MI) has occurred.[76,77] POC devices that measure blood cTn concentrations in <30 min would provide more efficient management of patients admitted suffering from chest pain. These devices would need to measure cTnI and cTnT with a coefficient of variation (CV) $\leq 20\%$ at the 99th percentile upper reference limit (URL) in order to reduce false positive and negative results.

The second opportunity is in sensors for detecting CSF. Currently, there is no POC sensor for giving immediate feedback on the presence of leaks during surgeries. The development of sensors for this application are at an early stage of development and much remains to be done in quantifying the sensors by calibration with ELISA and other measurements and establishing the measurement protocols and reproducibility. There is tremendous current interest in medical sensors that utilize semiconductor approaches and have excellent sensitivities and scalability.[78–87] This is an area that will grow rapidly as wearable and automatic data logging and transmission are incorporated.

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