Electrochemical Biomarker Sensor

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***Abstract*— The ability to monitor specific biomarkers in at-risk patients is extremely important in today's medical and healthcare systems. There are many sensing devices allowing accurate detection of such biomarkers, and this report reviews one called the electrochemical biomarker sensor. This sensor detects target analytes by converting electrode potential changes caused by chemical redox reactions into measurable electrical signals. In our analysis of this sensor, we first summarize the process of defining and identifying biomarkers, as well as qualify how their levels of concentration may be telling of a person's health condition. After we review and provide examples for the general classes of electrochemical biosensors, we also investigate the emerging fields of carbon nanomaterial biosensors and wearable biosensor technology. Both active fields reflect current state of the art developments in electrochemical biomarker biosensors.**

I. Introduction

The increasing prevalence of smart technology and materials has drastically changed the way we interact in our daily lives. The further development of this emerging technology can enable us to accumulate information to better understand our health and ourselves. Electrochemical biomarker sensors represent a crucial and exciting application that has the potential to revolutionize the health care industry.

Conventional sensing such as a blood test is a multistep approach that requires the invasive drawing of a patient's blood before it is analyzed. Electrochemical biomarker sensors represent an integrated non-invasive approach to sensing with advantages in its low cost, high sensitivity, and compatibility with microelectronics. A biosensor includes a sensitive biological element, the biomarker, that uniquely interacts with the desired substance such as enzymes, antibodies, nucleic acids, receptors, and more, and a transducer element that transforms the signal from the resulting interaction into a more easily measured and quantified signal such as an electrical signal with electrochemical sensors. Compared to traditional bench processes, biosensors can be both faster and smaller while detecting a wide range of phenomena. The level of health of an individual can be determined through measuring and processing a myriad of biological metrics. These biomarkers are important because they further open the door to preventative healthcare by providing relevant information immediately and addressing the issue before it ever becomes a problem. The flexibility of electrochemical biomarker sensors and advancements in biosensors materials and portability are significant topics of active research.

II. Compatible Biomarker Development for Sensor Applications

A biomarker is a measureable substance in an organism or environment that indicates some phenomenon relating to disease, health, infection, bodily information, or environmental exposure. Basic understanding of these biomarkers must be reviewed first to understand electrochemical biomarker sensors. Research on biomarkers has extended into many fields, and improvement on their design and application in measuring substances are continuously changing. There are many types of biomarkers used as indicators for electrochemical sensors to detect disease, environmental, and biological changes.

The biomarkers serve to improve biosensor detection of diseases or other undesired anomalies in a body. This section of the article will discuss various biomarkers used in biosensors applications. Biomarkers are good indicators used in a variety of field to signal certain phenomenon in which biosensors use to detect.

A. Transplantation Biomarkers Application

An example of biomarkers sensor application is in transplants. Biosensors along with sensitive biomarkers are used for immunological monitoring of transplant organs to detect changes and to avoid acute rejection and chronic allograft dysfunction [1].

The sensors used to sense these biomarkers for this treatment is a non-invasive biomarker that tries to predict graft rejection and dysfunction before possible onset of clinical symptoms [1]. This biomarker biosensor main goal is to detect possible manifestations or changes in the implants to enable timely treatment. The process below describes a sample for the development of biomarkers for these electrochemical biomarker sensors.

III. Biomarkers Development Process

For biomarkers that are used in the early detecting of cancer, genotoxicity, or other disease, certain criteria must be satisfied. The biomarker must be able to detect the disease, diagnose the disease, and evaluate response. The crucial idea here is to build a capable noninvasive biomarker for the biosensors to sense and notify doctors to cancer or disease at an early stage for possible preventive treatments.

To create biomarkers for sensors to detect cancer cells at early stages, the cancer needs to develop along advancing

deviations from the norm [2]. As for other disease detection, greater deviations from the normal cells make detection easier for biomarker type biosensor. This idea basically means that cancer cells must be biologically distinguishable and leave some biomarker signal for the electrochemical biosensors to detect the difference.

When developing biomarkers for cancer cell detection, there must be a classification of possible candidates of biomarkers associated with the disease under study [2]. In various other biomarkers, certain biological properties must be identified and be detectable by the biosensor for the biomarker to relay reliable data and information about the condition of the biological or physical environment [2].

A. Step to Transplantation Type Biomarkers Development

To develop most biomarkers, the following procedure is as followed. From biomarker discovery to clinical implementation [1]:

- Analyze biological sample
- Biomarker discovery
- Biomarker verification
- Biomarker validation
- Development of simple noninvasive lab test

1) Analyze Biological Sample

To start developing a biomarker certain characteristics that make the disease or change identifiable must be known. The biological sample and the disease or undesired trait is analyzed to identify the suitable biomarker.

In an example for cancer, the investigation of molecular signatures must be identified for the diseased tissue [2]. The diseased tissue's molecular signatures are then compared to the healthy tissue [2]. When comparing the biomarkers, they are assessed with both the contaminated and non-contaminated sample. This information and difference is then recorded for later development of suitable biomarkers for the sensor.

In DNA damage identification, biomarkers must be capable of identifying changes in the DNA structure [3]. Identifying DNA damage signal amplification has been shown to improve sensitivity detection for DNA anomalies and is used to construct biomarker sensor [3].

The main idea for analyzing biological samples is to obtain data for building biomarkers to detect the undesired anomalies in organisms or the environment [1, 3]. The properties used for the detection must have a large enough gradient or deviation from the healthy or normal environment. Without analyzing and comparing the healthy and unhealthy environment, biomarker biosensors made to detect the anomalies may not function properly if the distinguishing characteristics from normal and abnormal conditions are too closely related.

2) Biomarker Discovery

In the biomarker discovery stage, it requires identifying algorithms to distinguish differences in biomarker response to disease or environment [2]. This step employs extensive research and test to obtain data that will identify the best discriminating biomarker. The biomarkers normally outnumber the required investigated subjects. Due to the sheer volume of biomarkers, precise investigation is needed to exclude biomarkers detected by chance [2].

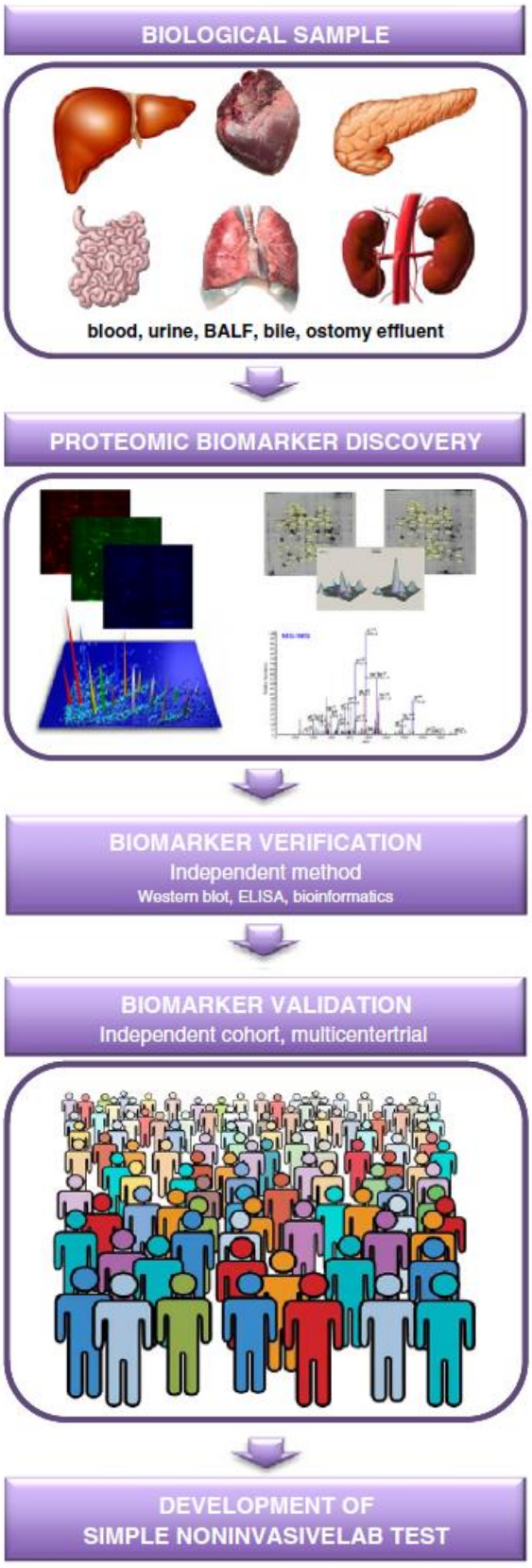

Fig. 1: Workflow schematic of biomarker discovery to implementation [1]

In most discovery of biomarkers, they are usually data driven and not reliant on biological hypothesis only [2]. This whole process involves narrowing down the biomarker suitable for biosensors to sense and provide the most informative data.

3) Biomarker Verification

In this step, biomarker verification is performed to make sure the biomarker is suitable for the sensors in identifying the anomalies and performing its task. Testes and experiments are performed to obtain data of the biomarker capabilities and its range and tolerance for accurate data report when detected by biosensors.

4) Biomarker Validation

In this step, biomarker validation refers to the evaluation of the biomarker's performance in detecting disease. This step also require researcher to find data on how early the biomarker can detect the anomalies before symptoms start showing. The biomarker is also assessed for its overall benefit compared to conventional diagnostic markers. It is important to note that some authors refer to verification and validation without distinction [2]. The basic idea of validation for biomarker is its ability for disease detection, detection time, and overall performance.

5) Development of Simple Noninvasive Lab Test to Approval

The biomarker at this stage is assessed through a rigorous trial for its benefit in comparison to usual care. The tests are randomized controlled trail used to assign participant to different trail [2]. Once tested, the new biomarker should exhibit the following properties. The biomarker must be suitable for clinical practice [2]. The biomarker should be robust and handle different test modifying factors thrown at it. Once in production, the cost should be moderate compare to conventional standards but still outperform the traditional biomarkers [2]. Overall, there should be improvement in the cost-benefit of the biomarker.

The diagram in Fig. 1: Workflow schematic of biomarker discovery to implementation shows further steps after the validation of the biomarker and how the process move from biomarker discovery to approval step.

All biomarkers should have a performance measurement to guarantee they are qualifying for internal use. Measurement of the disease-predictive value is needed to make sure the biomarker is qualified for internal usage [1, 2]. The test will provide information on how well the biomarker performs when detecting a disease prior to symptoms occurrence. This detection is strongly reliant on the disease incidence [2].

Once all these tested have been performed and biomarkers are verified, usage can then be approved of with the electrochemical biomarker sensors.

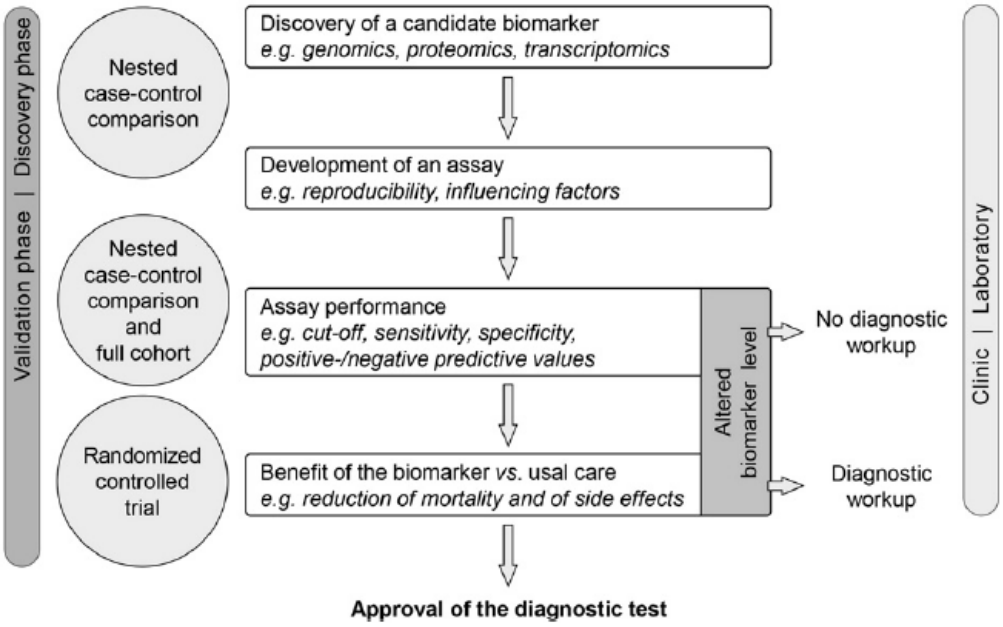

Fig. 2: Workflow schematic of how biomarkers are discovered, developed, and assessed [2].

IV. Type of Biomarker Sensors

There are many types of "electrochemical biomarker sensors" used to detect anomalies and phenomenon. A chemical sensor is an analytical device that detects analytes by combining a biological component with a physicochemical detector. The IUPAC definition of a biosensor is a chemical sensor device "that transforms chemical information, ranging

from concentrations of specific sample component to total composition analysis, into analytically useful signal".

These sensors usually have built-in components providing the necessary bio-reaction to convert substrate to products. A transducer that converts the information into electrical signal determines this reaction. The output signal of the transducer may be amplified for better signal by various types of amplifier circuits. This amplified information is processed and display or stored. The process of this procedure is depicted in the diagram below.

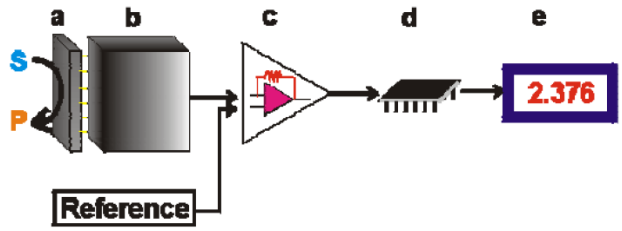

Fig. 3: Schematic of biosensor main components [12]

A. Sensors Detection Mode and Types

Electrochemical biosensors use current, electric potential, resistance, or impedance to help regulate changes and determine abnormal changes. These changes normally introduce biomarker indicators that biosensors could detect. There are various types of electrochemical sensors. Listed below are the various electro-analytical method used for different sensor:

- Potentiometric
- Amperometric
- Voltammetric
- Conductimetric
- Polarographic
- Impedimetric
- Capacitive
- Piezoelectric

This category of electrochemical sensors uses electro-analytical methods to detect the phenomena changes necessary for the sensor to response. This electro-analytical method is define as a class of analytical chemistry techniques used to study analytes by measuring the current or the voltage potential changes in an electrochemical cell containing the analyte. There are three main categories for electro-analytical methods and they are potentiometry, coulometry, and voltammetry. Potentiometry is the measuring of the electric potential. Coulometry is the measuring of the current over time. Lastly, voltammetry is the measurement of current when the electric potential is changing.

The list above shows major known electrochemical transducers used in biomarker sensors. The few sensor detection mode focused on this research are:

- Potentiometric
- Amperometric
- Voltammetric

The focus on these types of sensors is due to their quick and effective response. Electric current and voltage can be change instantaneously providing possible quick detection time if the sensors are built correctly. Measuring current and voltage have been done extensively in many fields making this the top choice. The potentiometric detection method will be the key focus in this article, because most of the topic that will be discussed in this article will be related to the potentiometric detection method.

Potentiometric Sensors –

A potentiometric sensor is a type of chemical sensor that is used to determine analytical concentration of analyte gas or solution. This type of sensor measures the electric potential between the electrodes in the solution against the reference electrode to obtain a potential difference. This process passively measures the electric potential between two electrodes without affection the subject being measure by much.

This type of sensor will also be discussed later on in the report. This technique will be incorporated into electrochemical biomarker sensors for the detection of bacteria and microorganisms [4].

Amperometric Sensors –

The amperometric sensors also determine and display the results using electric components and properties as well. The amperometric biosensors works slightly different by using current. The amperometric biosensors function by producing a current when the potential difference between two electrodes is changed [5].

Voltammetric Sensors –

In the case of the voltammetric sensor, these sensors can be used in the analysis of various organic and inorganic analyte. These voltammetric sensors function by measuring the current as the potential is varied. This data is related back to the analyte being analyzed.

An example of this type of electrochemical biosensor will be discuss in the nano-biosensor detection of genotoxins in water samples [6].

Conductimetric Sensors –

To monitor changes this type of sensors record information by measuring the electro-conductivity. The measurement may be done on the subject solution being analyzed. This technique requires and assumes that the material or solution being measure would have changing conductivity when certain phenomenon occurs. When the conductivity changes this

information is recorded and may be used to indicate certain phenomena. This process requires measuring the current directly or indirectly to determine the current pass so number of electrons could be determined.

Polarographic Sensors –

These types of sensors are a subclass of voltammetric sensors. The working electrode in this case is a dropping mercury electrode (DME) or a static mercury drop electrode (SMDE). The measurement process is similar to the voltammetric sensors.

Impedimetric Sensors –

This type of sensor involves measuring resistive and capacitive changes caused by the phenomena. This type of electrochemical sensor will not be dealt with in this current research report. The measuring of resistive and capacitive changes will provide the information of the effect this phenomena cause.

Capacitive Sensors –

This type of sensor involves measuring the change in capacity. This sensor will also not be discuss extensively in this research.

Piezoelectric Sensors –

This type of sensor measures the change in pressure, acceleration, strain, and force by converting the physical signal information into an electrical charge. These types of sensor are used in many fields but in this research, not much will be discussed about this type of sensor. This type of sensor becomes significantly more difficult to operate when it is in the nano-size scale. This sensor requires converting physical phenomena to electrical signal. In this research study, most of the researches are for biological phenomena so pressure, acceleration, strain, and force are not necessary information to determine the phenomena.

The term "electrochemical" refer to the field of electrochemistry. The focus on this research is electrochemical biomarker sensors. Electrochemical plays an important role in making the sensors detection method quicker and more efficient. Electrochemistry is the study of charge transfer phenomena. The idea is to qualitatively measure the electron transfer phenomena or related electro-properties and convert that information to sensor information and output the results. The field of electrochemistry includes a wide range of chemical and physical phenomena relating to many practical applications for analytical measurements.

With the understanding of basic sensors detection mode using potentiometric, amperometric, and voltammetric cleared, this research will now move toward providing examples of sensors. Most of the sensors discuss in this report are going to be electrochemical sensors using electrons flow as sensors indicators. The focus of this paper will be on electrochemical biomarker sensors but these sensors will use the detection method mention above in the background when sensing biomarker indicators.

V. Nano-Biosensor to Detect Genotoxins in Water Samples

There are many types of electrochemical sensors being develop to benefit humanity. One type of nano-biosensors being develop is the detection of genotoxins in contaminated water [6]. This type of experiment is done to guarantee that the water sample is safe to drink. Genotoxicity describes the chemical agents that could damage the genetic information within a cell causing cell mutations and other defects. This type of research is important as water contamination may affect whole cities if not maintain property. This technique will provide possible future techniques to detect genotoxicity in water samples.

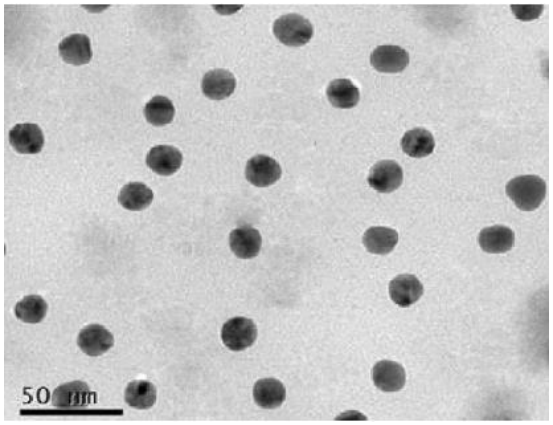

Fig. 4: TEM image of gold (Au).nanoparticles [9]

It is said that more pollutants exhibiting genotoxicity have invaded the environment and various water sources in the recent decades due to unmaintained waste disposal [6]. Lots of water contain genotoxic contaminates because of the unmonitored disposal of industrial effluents. When the industrial effluents are discharge into surface water they may be reused downstream by farmer for agricultural irrigation or by people as drinking water. Either way this exposes the person to possible health effect and possible damage to cell making them more likely to mutate and cause cancerous disease. This research will provide examples of possible benefit these electrochemical biomarker sensors will provide to society. To fight this genotoxicity issues the technique and electrochemical biosensor below does just this.

The technique this electrochemical DNA nano-biosensor employs is voltammetry [6]. This means that this genotoxicity sensor is a type of voltammetric sensor as described earlier in the sensor detection type section. The article presents a disposable electrochemical DNA nano-biosensor for quicker detection of genotoxic compounds in water pollution [6]. This

biosensor is prepare by immobilizing DNA onto gold nanoparticles and a monolayer of cysteamine gold electrode. The DNA and gold nanoparticle can be characterized by voltammetry. The type of voltammetry use in this case is cyclic voltammetry (CV). Cyclic voltammetry occurs when the electrode potential is ramped up linearly vs. time until it reaches a set potential. Once the set potential is reached the working electrode's potential ramp is inverted. The measure current can then be plot vs. the applied voltage to give the voltammogram trace. This will provide the necessary data for the analysis. On the other hand, the analyte and the immobilized DNA strand can be measured through the variation of the electrochemical signal using square wave voltammetry (SWV).

With the process described above this biosensor could detect the following genotoxic compounds: 2-anthramine, acridine orange, and 2-naphthylamine [6]. The detection limit for the following compounds varies when performing the genotoxicity analysis using this electrochemical sensor. The biosensor was used to test actual water sample as well to determine the varying contamination level. This biosensor is also compared to classical genotoxicity to confirm the capability and reliability of this particular electrochemical biomarker sensor.

The benefits of this sensor are its capabilities of detecting the carcinogens in drinking water and provide certain details about its effect. Current methods of detection use either gas (or liquid) chromatography or spectroscopy to identify the carcinogens [6]. This methods offer high possibility of identifying and quantifying the specific compounds with good precision and resolution.

However, this chemical measurement method using chromatography and spectroscopy does not provide information on the effects of the genotoxicity compounds because genotoxicity is a biological response [7]. These chromatography and spectroscopy test does not provide enough information relating to those responses so in this sense the biosensor could provide more information since it has DNA strand build into it that the genotoxicity compound could interact with [6].

The benefit these gold nanoparticle electrochemical sensors bring over classical methods comes from the ability to accumulate genotoxicity information with small amount of sample and uses biological interaction [7]. Classical methods such as chromatography and spectroscopy lack the ability to reflect the real effects of genotoxicity because of lack of biological response [5]. To obtain genotoxicity another method is used. Several classical methods include using bioassays to establish genotoxicity analysis. The genotoxicity bioassays can determine the effects of genotoxic compounds on a DNA strand [6]. These bioassays can provide many useful information regarding the genotoxic compound. The drawback of this classical method is the time and cost required to analyze the water sample for possible genotoxic compound [6]. The time require for the analysis can range from several days to weeks making information gathering and reporting slow and hazardous to individual already consuming the water sample within those few days or weeks. One article states that great progress have been made for the biosensors increased sensitivity and performance.

When building this voltammetric electrochemical sensor the most crucial step is the immobilization of the DNA strand [6]. This strand must be place and oriented carefully onto the surface of the electrode [6]. The placement of this immobilized DNA strand will greatly affect the accuracy, sensitivity, and selectively of the DNA electrochemical sensors [8].

The high surface to volume ration of this sensor makes assembling large volumes of DNA strands onto the electrode surface possible. This means that when used to test the water sample later there will be more reaction site for the water sample. This new design accuracy, sensitivity, and selectivity may change with higher DNA surface immobilization density [6].

Another type of sensor detection uses silver nanoparticles in its design [9, 10]. This particular silver nanoparticle biosensor will not be discuss extensively in this paper. There are still various many other types of electrochemical biomarker sensors to be discussed.

With this particular sample out of the way, there are still many other electrochemical biomarker sensors to discuss. Some other examples to be discussed include carbon nanomaterial based biosensors and wearable biosensors.

V. Carbon Nanomaterials

As described previously, electrochemical biosensors are based on the implementation of transducing devices that convert information from chemical events into measurable electrical signals. As chemical reactions, such as electron redox reactions, occur in a biological system, these biosensors measure fluctuations in voltage or current and proportionally relate the differences to the concentration changes of the target analyte. Such methods are achieved by using two-electrode constructs (one reference electrode and one source electrode) that allow the measuring of electric potential and current across a conducting medium. It is across this medium where electron transferring due to chemical reactions facilitated by enzymes and reagents instigates the changes in potential that signal the sensing of analyte.

The ultimate goal for researchers designing sensor devices is simple: improve sensitivity, accuracy, stability, repeatability and response time. For electrochemical sensors,

that can be achieved by either more reliable techniques of facilitating the biochemical reactions, or by improving the electrical efficiency of the medium connecting the electrodes. The latter is an active goal of research and relies heavily on the consideration of the material choice used to construct the transducing elements. When talking about current electrochemical biosensor transducer materials, one has to undoubtedly discuss the emergence of carbon based nanomaterials.

Carbon based nanomaterials are novel and advantageous for many different types of sensing devices because of their favorable physical qualities. The most impactful of these qualities include high electrical conductivity, high strength, dimensional flexibility, chemical stability, and are acceptable with respect to biocompatibility [11]. These particular material characteristics allow for sensors that generally have higher sensitivities and lower detection limits than conventional counterparts.

VI. Carbon Nanotubes & Graphene

Of particular interest are two specific forms of carbon nanomaterial structures, both of which are surprisingly simple. The first is called a carbon nanotube which, as its name suggests, is a tube of carbon atoms created by rolling up one or more layers of carbon atoms.

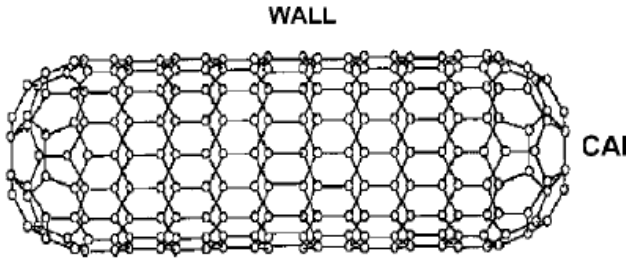

Fig. 5: Structure of a single-walled carbon nanotube (capped) [12]

These carbon nanotubes can have multiple layers by rolling up multiple stacked carbon layers, but are often utilized as single-walled carbon nanotubes (SWNTs) as shown in Figure 1. The diameters vary between 0.4nm and 2.5nm, and the lengths can vary greatly to accommodate design needs [11]. One of the novelties of carbon nanotubes is that they have the ability to exhibit large length-to-diameter ratios that can reach up to 100 million to 1. For this reason, carbon nanotubes are sometimes referred to as 1-dimensional "nano-wires" that can be used to physically attach one nanosized object/feature to another [13]. It should be noted that carbon nanotubes can also be multi-walled, meaning that they are composed of the rolling of multiple stacked carbon layers. In contrast to single-walled carbon nanotubes, the diameters of these thicker walled nano-tubes can range from 2-100 nm [14]. These multi-layered tubes are superior to single-walled nanotubes in some instances, and demonstrate how the dimensions of carbon nanotubes can be tailored to meet certain design requirements.

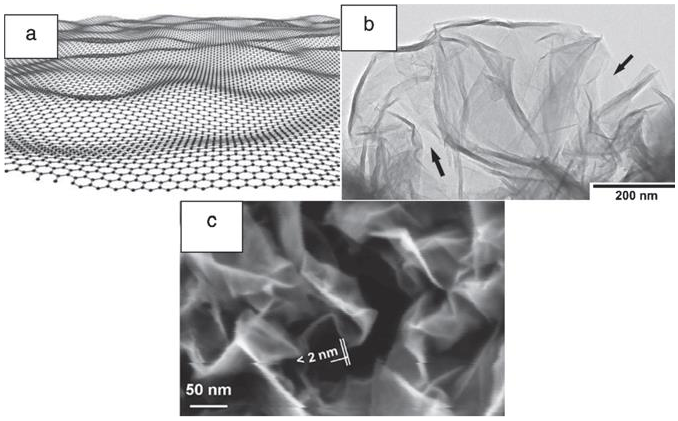

Fig. 6: (a) Structural model of graphene, (b) TEM image of graphene, (c) SEM image of graphene [15]

The second notable form of carbon nanomaterial structure is called graphene. This carbon structure is a monolayer of carbon atoms, and is the structure that one would get by unrolling and flattening a SWNT. As they are only one carbon atom thick and often exhibit large surface areas, graphene sheets are described as 2-dimensional structures.

VII. Advantages for use in Biosensors

The significance of these two types of carbon nano-structures lies in their general physical properties as carbon materials, as well as their individual dimensional uniqueness. Both exhibit extremely high electrical conductivities (much higher than gold, silver, copper or other metals) as well as high mechanical strengths (much higher in proportion to steel). High electrical conductivities allow for faster conduction of electrical signals due to high electron mobility, hence allowing more rapid transduction processes in electrochemical biosensors. High mechanical strengths allow for the construction of more robust structures that can survive perturbations from active environment conditions that may be present in dynamic biological systems.

Individually, their geometric dimensional properties make them useful in building biosensors. Graphene's large surface area is ideal because it allows for analyte sensing over larger target areas. The larger surface area also means that more enzymes can be attached to the surface between the reference and working electrodes, meaning that more analyte can be sensed at a given moment. As will be seen in a later example, the concentration of detection enzymes on the conducting medium between the electrodes actually plays an important role in determining the effectiveness of the sensor. Carbon nanotubes' instrumentation as 1-dimensional electron transferring "nano wires" allows for analyte sensing across

long distances and through tight spaces where graphene may not be able to reach. The linking of these carbon wires between electrodes and their attachment to enzymes gives a more controlled sensing method than graphene, which almost always covers a wide open space.

Overall, these two types of carbon nanomaterial structures are valuable with respect to their superior physical properties and their unique dimensional.

VIII. Carbon Sensor Examples

Over the past decade, there have been a myriad of studies presenting different ways of designing electrochemical biosensors that use some form of graphene or carbon nanotubes as transducer materials. These sensors have been designed to detect many types of biomarkers such as cholesterol, lactate, dopamine, etc. [15] . Carbon nanomaterial sensors have even been designed to selectively detect live microorganisms like bacteria [16]. Since it would be impractical to describe in detail a wide range of example studies, this section will focus on examples characterizing the design of one particular type of sensor that reasonably represents the field as a whole. That sensor, which happens to be one of the most important electromechanical biomarker biosensors, is the glucose electrochemical sensor.

Glucose Oxidase Electrochemical Biosensor

Glucose electrochemical biosensors account for the majority of the medical sensor industry in terms of both industrial market and research. Diabetes is a prevalent condition affecting millions of lives and is caused by a metabolic disorder resulting in the deficiency of insulin and hyperglycemia. These deficiencies are reflected by abnormal blood glucose concentration levels higher or lower than the normal range of 80-120mgdL^{-1}. The disease affects people worldwide and is a leading cause of death and disability [3]. For this reason, there is an ever growing need for technology allowing for the perpetual monitoring of glucose blood level in diabetics. Active sensor research is working to address this need by designing easy-to-use electrochemical sensors that can help a diabetic track his or her blood glucose concentration levels effectively and conveniently throughout his or her daily life. The main attributes of an ideal glucose sensor are high sensitivity, selectivity and portability, all while using only the smallest blood sample possible.

In relation to carbon nanomaterials, one of the methods attempting to design newer, more efficient glucose sensors involves the use of single-walled carbon nanotubes as the transducing element between a working electrode and a reference electrode [10].

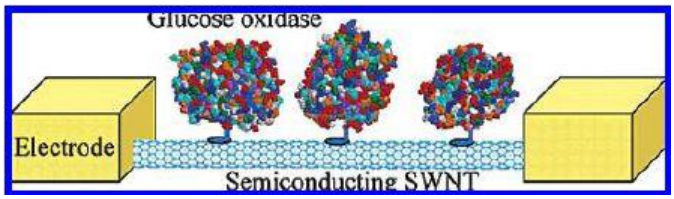

Fig. 7: Carbon nanotube (CNT)-based transistor for biosensing of glucose [13] (JW,2008)

In this sensor, which is depicted in Figure 2, the carbon nanotube acts as an electrical connector between gold electrodes. The carbon nanotube is coated with glucose oxidase (GOx) enzymes, which are immobilized on the nanotube surface. The GOx enzyme is used to react with incoming glucose molecules and catalyze the oxidation process. As these oxidation reactions occur and the number of glucose substrate increases on the surface of the single- walled carbon nanotube, the conductance of the GOx-coated nanotube changes accordingly. The measured change in conductance of semiconducting carbon nanotube allows for sensing of glucose concentration.

The previous example of an electrochemical glucose biosensor utilizing carbon nanotubes was one theorized and preliminarily tested back in 2003. Since that time, many recent developments in these types of carbon nanotube glucose sensors have been achieved, leading to further fabrication and testing methods. A prime recent example is demonstrated in an analytical study Pourasl et al [17]. In this study, a glucose sensor nearly identical to the one shown in the previous example is mathematically modeled in order to compare experimental performance results with predicted results. The modeled sensor is shown in Figure 4 and is composed of gold or chromium source and drain electrodes, a carbon nanotube source-to-drain connection, and a GOx layer on the carbon nanotube surface.

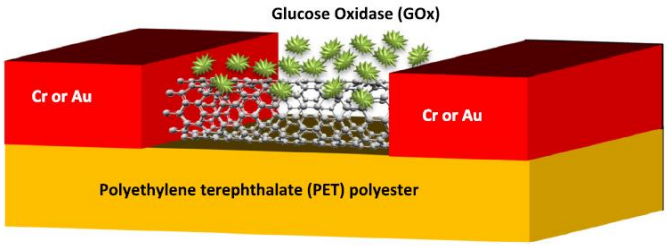

Fig. 8: Glucose sensor composed of gold or chromium electrodes, a layer of GOx biomolecular assembly, and a single-walled carbon nanotube in the form of FET [17]

The resulting FET electrochemical biosensor device was modeled using a charge-based carrier velocity model, as opposed to a noncharge-based analytical model using a using the surface-potential based analysis method. After using known physical properties of the device materials such as carrier mobility and dielectric constant to compute drift velocity parameters, the model is able to study the effect of glucose concentration on the current and voltage

characteristics of the carbon nanotube FET. The basis of formulation of this model relies on the simulation of the direct electron transfer that occurs in the actual glucose sensing mechanism. This mechanism is the oxidation of β-D-glucose to D-glucono-δ-lacetone and hydrogen peroxide that results from the catalyst reaction of GOx. This mechanism can be summarily represented by the following equations [17]:

$$(\beta - \mathrm{D} - \mathrm{glucose}) + O_2 with\ GOx\ enzyme \rightarrow (\mathrm{D} - \mathrm{glucono} - \delta - \mathrm{lacetone}) + H_2O_2 \quad (1)$$

$$(\mathrm{D} - \mathrm{glucono} - \delta - \mathrm{lacetone}) + H_2O \rightarrow D - gluconate^- + H^+ \quad (2)$$

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e \quad (3)$$

The transferring of electrons to the carbon nanotube FET by this oxidation event is quantifiably measurable by the changes in drain voltage and current, and by extension, the concentration of glucose. Glucose concentrations from 2-50mM were modeled and the calculated drain current and drain voltage data were compared to the experimental results. The comparison is represented graphically in Figure 5 for each testing glucose concentration.

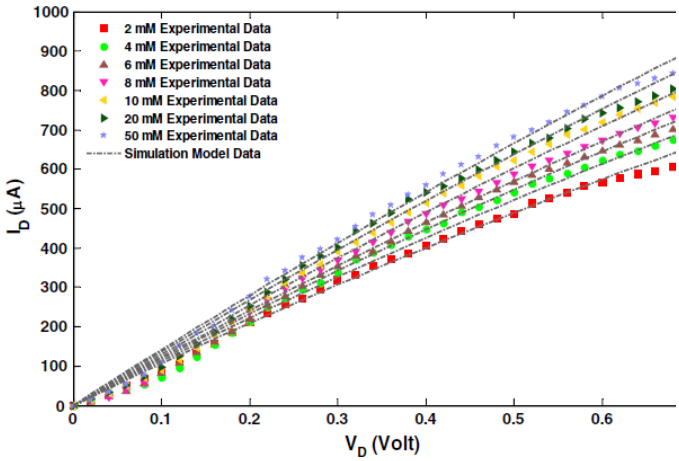

Fig. 9: I-V Comparison between experimental and modeled results for the carbon nanotube FET biosensor for various glucose concentrations [17]

As can be seen in Figure 5, the modeled results follow the experimental results quite closely. Statistical RMS error analysis is shown in Table 1 to mathematically verify that the modeled and experimental results vary by a relatively small margin. No normalized RMS error value exceeds 13%, which is a promising result.

Table 1: Error analysis between the simulated and modeled data for various glucose concentrations [17]

Glucose (mM)	Absolute RMS errors	Normalized RMS errors (%)
0 (with PBS)	19.24	5.66
2	57.55	12.22
4	49.05	9.75
6	59.47	11.23
8	53.99	9.80
10	55.60	9.53
20	69.18	11.17
50	75.07	11.60

Of course, more analytical trials need to be carried out; this study presents only one recent example. However, what the two previous case studies show is that electrochemical glucose biosensors using carbon nanotubes are of active interest, and have been so for at least the past decade.

Graphene has also been used in combination with oxidase to formulate a glucose electrochemical biosensor. In a study by Unnikrishnan et al. glucose oxidase was implanted onto the surface of sheets of reduced graphene oxide (RGO) to form a RGO-GOx biocomposite film. This film was produced by first immobilizing the glucose oxidase enzymes on mechanically exfoliated graphene oxide, and then using a solution to reduce the graphene oxide. This short process is effective and a rare simply solution to immobilizing enzymes efficiently onto electrode surfaces. After producing the RGO-GOx biocomposite films, different levels of glucose were measured using amperometry. The particular amperometry method used in this study utilized a rotating disk electrode (RDE), a technique that involves the convective mass transport of reactants and products at the electrode's surface. This artificial means of inducing analyte flux onto the electrode surface is a common method of testing the response mechanisms of redox reactions. The electrode in this case was spun at a constant 3000 RPM to promote good mixing of the analytes.

In testing the RGO-GOx film with the rotating disk electrode setup, performance in terms of glucose detection was based on the monitoring of the amperometric responses produced by the reduction of oxygen while gradually increasing the concentration of glucose. Since it is the reduction of oxygen level that acts as the measuring stick for glucose concentration in the vessel, careful attention was given to monitoring the atmospheric O_2 level of the experiment vessel. If the vessel is left open to the air, the loss of O_2 into the atmosphere caused by the spinning agitation of the rotating disk electrode causes the current to drop even when no glucose is added. This is depicted in inset (a) of Figure 4, where it can be seen that the current drops steadily

over time. This steady decreasing in current is obviously not ideal, and inset (b) of Figure 4 shows the amperometric response of increasing the glucose concentration when the vessel is left open to the atmosphere. In order to remedy this undesirable effect, the vessel was sealed and the O_2 concentration was kept saturated. The amperometric response to gluose concentration increases was again performed for this closed vessel condition, leading to the data seen in the main plot of Figure 7. Glucose was added every 50 seconds and as can be seen by the sharp decreases in current, the amperometric response to the increased glucose concentration was rather quick (> 5seconds). This data along with the data from Figure 4 inset (c) show that the RGO-GOx film was rather effective at detecting changes in glucose concentration, as the controlled linear change in glucose concentration in the vessel resulted in a fairly linear change in current.

This study also went on to show that this RGO-GOx film was also effective in detecting glucose even in the presence of other biomolecules, such as dopamine. Often, sensor readings can be caused to give false readings when foreign molecules are present to interfere with the target analyte. This is an issue that must be considered with most biomarkers, as complex biological environments are rarely isolated to only include the particular biomarker being targeted. This is especially true for glucose sensors since blood obviously contains many biomolecules other than glucose. Fortunately, from the trends in Figure 8, we can see that this was not the case for this sensing biocomposite film. As can be observed, the current only decreased when 1mM glucose amounts were added to the experiment vessel. When dopamine (at 150s), uric acid (at 200s) or absorbic acid (at 300s) were added to the solution, the current stayed constant, thus indicating that the sensor current reading remained unaffected by the introduction of the foreign molecules.

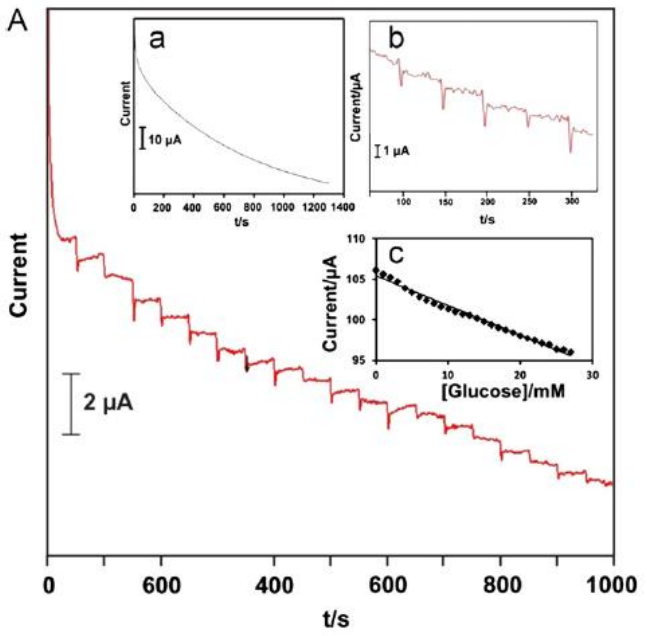

Figure 10: Amperometric current-time response for the addition of different glucose concentrations into O_2 saturated PBS, inset (a) shows the decrease in current with time in the absence of glucose, inset (b) amperometric response when the solution is exposed to O_2 from the atmosphere while glucose concentration is increased, inset (c) plot of glucose concentration vs current in the linear range [18]

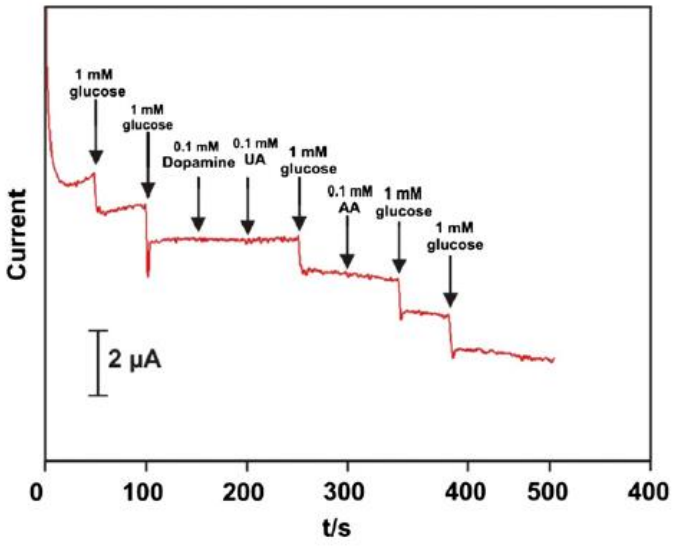

Fig. 10: Amperometric current-time response of RGO-GOx modified rotating disk GCE for glucose concentration variation in the presence of interferring species in O_2 saturated PBS [18]

Overall, the RGO-GOx film glucose sensor proved to be highly responsive to changes in glucose concentrations as well as highly selective when exposed to foreign molecules. Tests were also run to assess the storage and stability of the sensor when exposed to adverse temperature conditions. To perform this test, the RGO-GOx was kept in PBS at 4ºC and pH7 for 20 days. Current through the film was recorded periodically and it was found that the sensor retained 93.4% of its initial response. Further testing showed that it still retained 81% of its initial response after 50 days of the described temperature and pH conditions. The exhibited stability is very good, and indicative of favorable biocompatibility between the RGO sheet and the GOx enzymes immobilized on its surface. These desirable sensing qualities of high sensitivity, high selectivity and consistent stability, as well as the ease of fabrication, give one positive example supporting the advancement of graphene utilization in glucose sensors.

IX. Future & Limitations

Although active research has yielded a number of examples utilizing graphene and carbon nanotubes in electrochemical sensors with positive results, there are still a number of hurdles that prevent the marketability of these devices. The prior sensor examples are only evidence of lab

success and not real work market success, and that is no coincidence. The ability to integrate these specialized materials into industry standards is still a major challenge.

One of the main obstacles is reliability of manufacturing large usable quantities of these carbon nanomaterials, especially with respect to graphene. Although many depictions of graphene in scientific papers show perfectly flat sheets of carbon, this is not usually the case. Often, the graphene sheets are wavy and scrolled because that is their natural state of lowest energy. This fact is non-ideal especially when considering biomarker biosensor designs that require the immobilization of enzymes onto the surfaces of graphene sheets. If the surface has tendencies to fold on and around itself then there is less surface area for embedded enzymes to react with and sense the target analyte.

There are a number of manufacturing methods for graphene that include the reduction of graphene oxide, epitaxy, carbon nanotube slicing, metal-carbon melts, mechanical exfoliation of graphite, and chemical vapor deposition (CVD). The most promising methods are probably the latter two processes. Mechanical exfoliation of graphite is known as the "scotch tape" method and is one of the earlier and most simple methods [19]. Bulk pieces of raw graphite are delaminated gradually, layer by layer, until only the single layer graphene sheets are left. This process produces high quality graphene (known as pristine graphene) that would be useful in the fabrication of reliable sensors. The drawback of this method is that it only produces small quantities of graphene at a time. Bulk production using this process at present would be costly both in time and money. More recently, researchers have been producing graphene using CVD. This application is relatively new, and has been shown to produce large amounts of graphene with okay quality.

As it is today, cost-effectively producing high quality graphene for the production of reliable sensors on a large scale is not yet a reality. Carbon nanotubes face a similar situation with respect to manufacturing resources. The issue with carbon nanotubes is that their mass production results in tubes that vary greatly in length and level of impurities. These impurities, which may include metal ions, can significantly alter the electrical properties of the individual carbon nanotubes [19]. For this reason, selection of appropriately conditioned carbon nanotubes for biosensor application requires tedious screening methods that only select nanotubes of a desired length and purity. Obviously, this selection process can be costly both in time and money when thousands of carbon nanotubes must be sorted through. Until these issues can be resolved, mass production of biosensors using carbon nanotubes is not a viable reality.

In the end, the use of carbon nanomaterials in electrochemical biosensors is still a relevant area of research. The electrical, mechanical and dimensional properties of carbon nanomaterials such as graphene and carbon nanotubes are too extraordinary to overlook. As demonstrated by the abundance of research into their application as biosensor transducer materials over the past decade, it would be safe to say that researchers will continue to investigate means of furthering the technology. Although marketability is currently stunted by poor cost-to-benefit ratios due to difficulties in mass production of the carbon materials, there are a number of industry oriented groups attempting to resolve manufacturing shortcomings. For example, Graphene Frontiers, a company developed through the University of Pennsylvania, was awarded a $750,000 NSF grant in September of 2013 to further develop their "Roll-to-Roll" method of mass producing graphene. Their method can supposedly produce meter-long sheets of high quality graphene, which of course would be significant for applications to industry manufacturing. Success and further funding of companies like Graphene Frontier may make carbon nanomaterial electrochemical biosensors industrially relevant in the near future.

Until that time, electrochemical biomarker biosensors are being developed for various other interesting applications that will likely change our daily lives in the very near future. One such application has been the development of wearable biosensor technology.

X. Wearable Electrochemical Biosensors

One of the most interesting developments of electrochemical biomarker sensors is its integration into an individual's everyday life. Rather than being considered a distraction or inconvenience, wearable biosensors integrate seamlessly while providing valuable and novel real time information on one's health and well-being. These robust sensors integrated onto clothing allows the user to receive direct information without reducing comfort or ease of use. The range of applications of the sensors are quite varied and include measuring physical parameters such as heart rate, respiration, blood oxygenation, blood pressure, temperature, motion, brain activity, and more. Using microelectronics and wireless technology, the data can be easily collected, transmitted, and processed to provide crucial information on the user. The growing acceptance of gadgets and the wear-and-forget ease of use, drives the development behind such biosensors. This data, used in conjunction with standard forms of testing, can provide a more complete picture of the user's health that includes trends over time to improve diagnosis. Overall fitness can be monitored as well as the opportunity for increasing preventative measures early on. The success of wearable biosensors relies on developing a technology platform that is durable enough to withstand continuous mechanical wear and physical activity while maintaining consistently accurate measurements in a non-intrusive manner.

XI. Clothing Based Biosensors

Integrating electrochemical biomarker sensors onto clothing fabrics including wool, cotton, nylon, polyester, and others is a more traditional non-invasive approach for developing wearable biosensors. Fabrication of such sensors relies on the popular screen printed electrodes technique which is easily scaled for mass production while retaining its low cost, robustness, and performance. Using specially designed patterned mesh-screen stencils, various layers of conductivity, bio catalytic functionality, and insulation are printed in a tightly controlled environment and then processed into working sensors. The stamp transfer electrodes technique, where each layer is transferred via reliefs, is also widespread due to its conformal ability to work on non-planar surfaces which is crucial to expanding the range of wearables. In addition to fabrication, it is also critical to have flexible electrodes where the device performance is unaffected as it is continually deformed. Though small deformations and bends result in negligible changes, 180 degree pinches suffer from reduced accuracy and cracks but do not result in device failure. The changes in the electrochemical response are the result of differing electrical properties such as resistance and varying enzyme sensitivity due to exposure. The enzymatic response normalizes after a few cycles, but the overall electrochemical response to stress presents a large challenge to wearable sensors. These clothing based wearable devices can be further defined into two categories, one where they monitor the user's health and one where they monitor the environment around the user. Internal looking biosensors have been the norm, yet wearable sensors can further develop the category to improve one's health, fitness, and performance. External looking wearable biosensors are a novel and largely unexplored application. With breathable and water-proof characteristics, they are capable of identifying potential hazards such as air quality and contaminants to promote the user's safety while increasing the definition of available information.

XII. Epidermal Based Biosensors

Another level of integration is the application of electrochemical biomarker sensors directly to the skin rather than to clothing. The direct epidermal integration allows for optical techniques and components unsuited to fabric based sensors to take advantage of chemical constituents residing on the surface while remaining non-invasive. This greatly expands on the available dimensions and information to further insight into the user's overall health status, but epidermal integration remains difficult to achieve due to incompatible elasticity between the substrate and skin. To achieve full conformity to the irregular contours characteristic of human anatomy, electrode systems based on transfer tattoos and thick-film fabrication yields compatible integration with the skin. To increase their durability due to significantly increased wear, carbon fiber segments are often added to the sensor giving it increased resistance to deformations including pinching, stretching, and twisting. This also provides an interlinked conductive backbone that improves electrochemical performance.

Wearable electrochemical biosensors represent a promising research field that would provide unparalleled information access to the benefit of the individual's health, fitness, performance, and safety. Integration of these sensors onto fabrics and the epidermis has remained challenging while retaining reliability and sensitivity hampered by stress. Overall electronic processing, communication, and power concerns of sensors are also being further developed and solved. Nevertheless, these wearable electrochemical biosensors provide novel real time sensing capabilities whose applications and impact for the general population has yet to be fully realized.

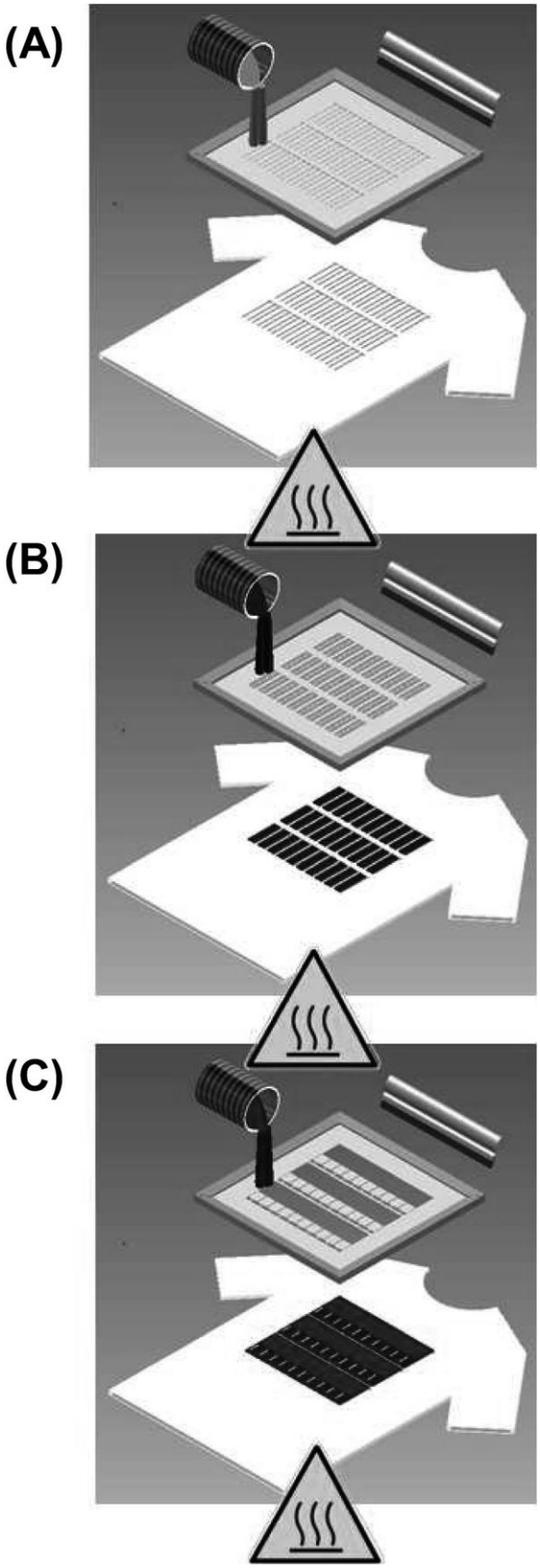

Fig. 11: Steps for screen printing electrochemical biomarker sensors on textiles.

XIII. Tattoo-based Potentiometric Ion-selective Sensors for Epidermal pH Monitoring

The integration of biosensors to the epidermal layer has resulted in a rethinking of the traditional potentiometric ion selective electrode. The conventional electrode relies on a membrane electrode, a reference electrode, an internal solution, to ensure a stable and sensitive response, and rigid substrates. These requirements pose significant limitations to the sensor design and layout which can only be mildly mitigated by eliminating the complex internal solution at the cost of accuracy or fabricating with flexible plastics. Epidermal sensors on the other hand are highly conformal maintaining a compact signature while preserving its sensitivity. By implementing screen printing and transfer tattoo fabrication techniques reinforced with carbon fiber to improve stress resistance, the development of epidermal solid contact based ion selective electrodes is achievable. Polyaniline based solid contact ion selective sensors are screen printed onto tattoo base paper with carbon, Ag/AgCl, and insulator inks before being transferred onto the desired substrate. This results in a tattoo based biosensor that is capable of measuring a wide range of pH levels while tolerating mechanical stress for various deformations and preventing skin irritation. Its design and location on exposed skin allows for direct and instantaneous pH monitoring correlated to perspiration and indirectly to respiration.

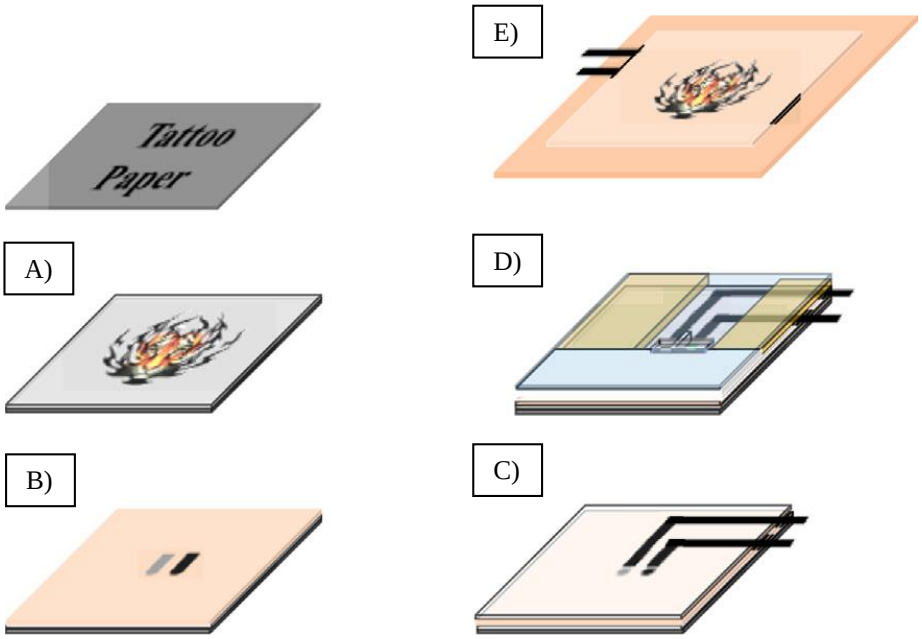

Fig. 12: Steps for the fabrication of the Na-tattoo sensors. A) Screen-printing an insulator layer on tattoo paper with design B) Skin colored ink is coated and two electrodes are printed C) Another insulator layer is coated defining electrode area and contact points D) Electrodes modified with membrane biomarker solutions E) Na-tattoo sensor is ready for application

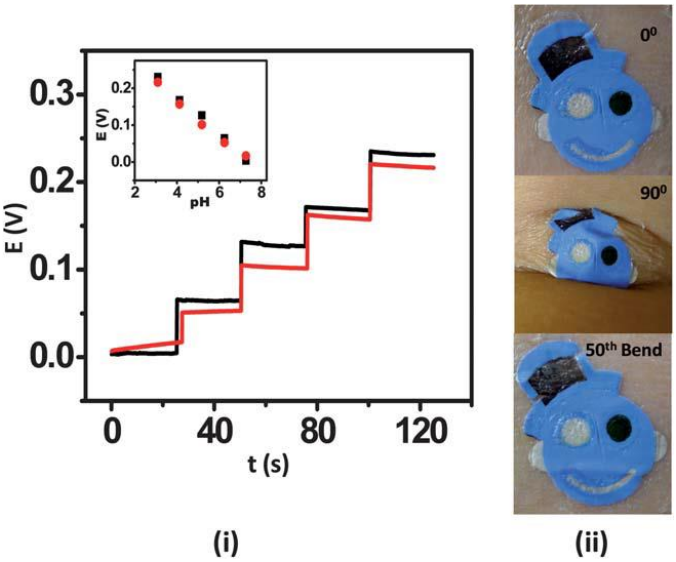

Fig. 13: The effect of bending mechanical stress on the pH-tattoo response signal.

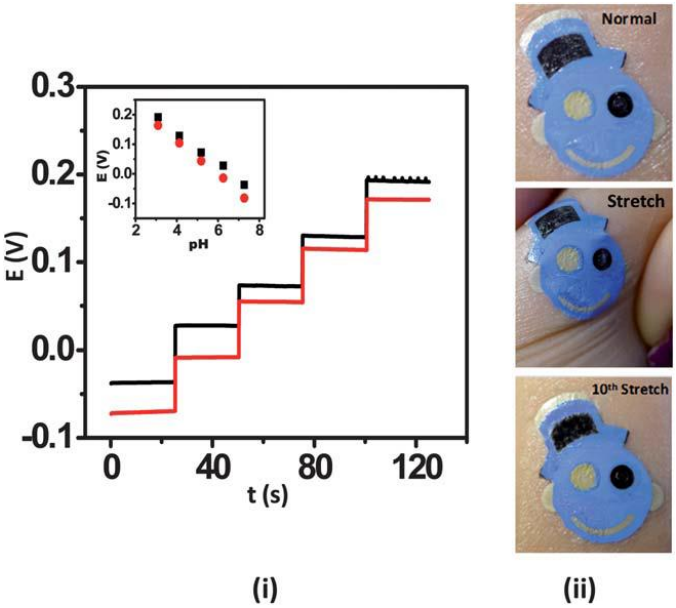

Fig. 14: The effect of stretching mechanical stress on the pH-tattoo response signal.

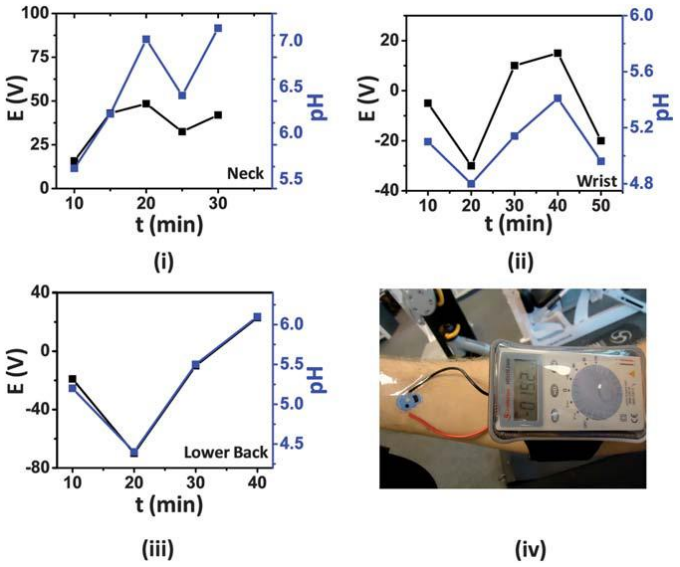

Fig. 15: The comparison between the tattoo sensors (black) and conventional pH meters (blue) on the neck (i), wrist (ii), and lower back (iii). The setup (iv).

In addition to measuring the pH, similar epidermal devices can also be calibrated to measure sodium, ammonium, and lactate concentrations. Sodium, a biomarker for electrolyte concentration, can be used to identify specific electrolyte levels to optimize its replenishment and prevent complications such as Hypoatremia from occurring. Electrolytes are essential as they regulate water balance, pH, and osmotic pressure within the human body. Ammonium, a biomarker for an individual's metabolic state, can also be used to observe for liver functions and hepatic disorders. Lactate, a biomarker for tissue oxygenation, is produced by anaerobic respiration during physical exertion and also provides valuable athletic performance data that would otherwise be obtained through invasive blood draws

XIV. Integrating Wearable Biosensors

The pairing of wearable biosensors to wireless technology allows data to be sent immediately, which has the potential to drastically change healthcare. Rather than having to examine a patient's history, continuous coverage of the patient can be achieved instead. The data can be sent via a short range transmitter from the biosensor to a smart phone which then communicates with a remote doctor or health professional. This level of connectivity not only reduces the amount of time it takes to respond to health conditions, but also facilitates preventative healthcare by being able to recognize and address complications at an early stage where more options are available and effective. With accurate and persistent information, connected wearable biosensors can significantly change the status quo and tackle problems before even symptoms are experienced.

XV. Epidermal Biofuel Cell

With the integration of so many functions however, a viable power source for the biosensor is required. To maintain the primary advantages of an epidermal biosensor, its power source must also be equally discrete. The use of battery is possible but would add additional upkeep for the user and is less attractive than a self-sustained device. Harvesting the mechanical motion generated by the human body with piezoelectric and similar generators is a possibility but could also be a limitation in where the biosensor can be placed. Instead, a novel approach to extract the user's biochemical energy can be applied. Using lactate, the very same biomarker that the biosensor is designed to detect, sufficient energy can be continuously generated. The lactate dehydrogenase enzyme catalyzes the selective oxidation of lactate to pyruvate which converts nicotinamide adenine dinucleotide from NAD+ to NADH with the mediator tetrathiafulvalene (TTF) carbon nanotube composite. The stable biofuel cell system is able to generate sufficient power is a way that makes it uniquely appropriate for biosensing while maintaining its durability.

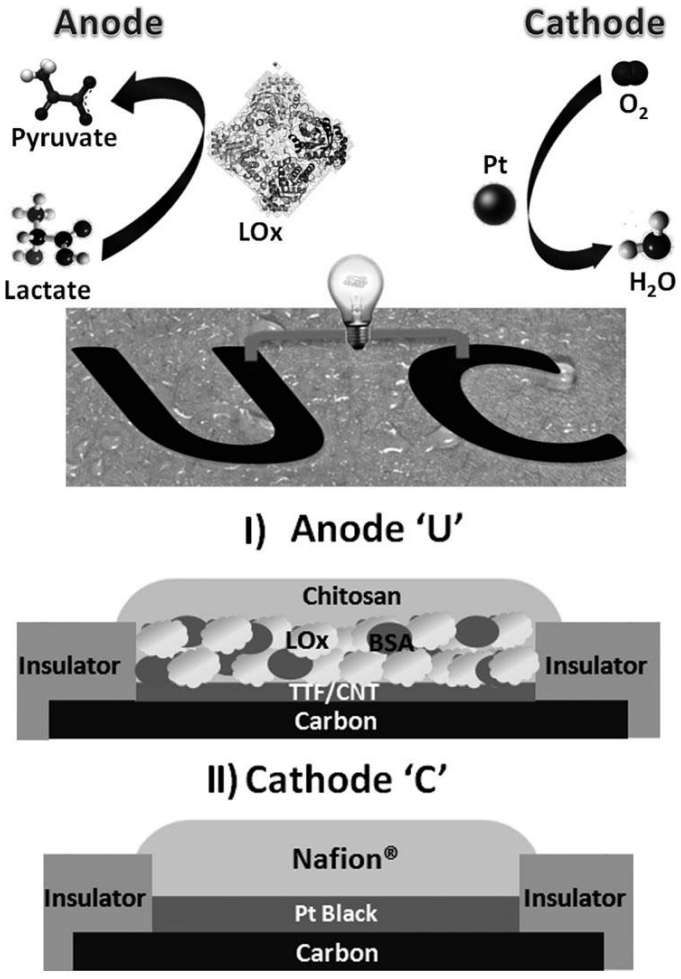

Fig. 16: Example of the epidermal biofuel cell with its anode and cathode components.

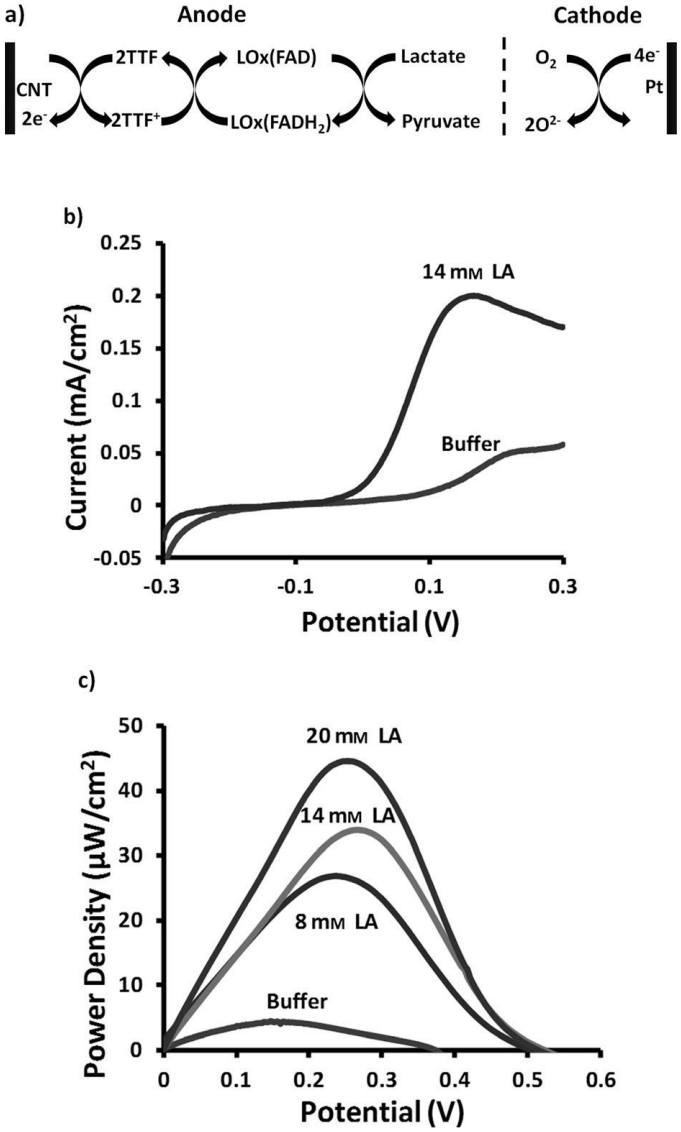

Fig. 17: a) The reduction-oxidation reactions that drives the epidermal biofuel cell b) Polarization curves in the absence and presence of lactate at 14mM concentration with 0.2M Mcllvaine buffer solution c) Power density of the epidermal biofuel cell at varying lactate concentrations.

XVI. Conclusion

With the continuous advancement of electrochemical biomarker sensors society can benefit greatly from extremely cheap and highly sensitive sensors. Some of these sensors are still in development but they still hold great potential in the near future. Electrochemical sensor detection capabilities will improve with better biomarkers used as indicators. Once the different detection type of sensors is understood, picking out sensors type becomes crucial. As shown in this report, biosensors can be developed using gold nanoparticles or graphene and as well as many other types of techniques. These electrochemical biosensors could even be built on wearable materials. This goes to show that electrochemical biosensors hold many possibilities in future applications.

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