This article has been accepted for publication in a future issue of this journal, but has not been fully edited. Content may change prior to final publication. Citation information: DOI 10.1109/RBME.2017.2706661, IEEE Reviews in Biomedical Engineering

Stability of Enzymatic Biosensors for WEARABLE APPLICATIONS

Apurva Sonawane[1], Pandiaraj Manickam[1], Shekhar Bhansali, *Member, IEEE*

***Abstract*—Technological evolution in wearable sensors is accounting for major growth and transformation in multitude of industries ranging from healthcare to computing & informatics to communication and biomedical sciences. The major driver for this transformation is the new-found ability to continuously monitor and analyze the patients' physiology in patients' natural setting. Numerous wearable sensors are already on the market and are summarized. Most of the current technologies have focused on electro-physiological, electro-mechanical or acoustic measurements. Wearable bio-chemical sensing devices are in their infancy. Traditional challenges in biochemical sensing such as reliability, repeatability, stability, and drift are amplified in wearable sensing systems due to variabilities in operating environment, sample/sensor handling and motion artifacts. Enzymatic sensing technologies, due to reduced fluidic challenges continue to be forerunners for translation into wearable sensors. This paper reviews the recent developments in wearable enzymatic sensors. The wearable sensors have been classified in three major groups based on sensor embodiment and placement relative to the human body: (i) On-body, (ii) Clothing/textile-based biosensors and (iii) Biosensor accessories. The sensors, which come in the forms of stickers, tattoos are categorized as on-body biosensors. The fabric-based biosensor comes in different models such as smart-shirts, socks, gloves and smart undergarments with printed sensors for continuous monitoring.**

***Index Terms*— electrochemical wearable biosensors, amperometry, enzyme stability, shelf-life, analyte.**

A. Sonawane, P. Manickam, S. Bhansali are with the Department of Electrical and Computer Engineering, Florida International University, Miami, FL 33174 USA (e-mail: asona003@fiu.edu; pmanicka@fiu.edu; sbhansa@fiu.edu). A. Sonawane, P. Manickam contributed equally to this work. Corresponding author: Shekhar Bhansali (email: sbhansa@fiu.edu)

I. INTRODUCTION

Wearable biosensors offer an endless range of potential ways to provide terms of timely diagnoses and to monitor the efficacy of treatments. Being able to track personal health metrics, measuring and maintaining patients' drug levels with great accuracy, maintain accurate measurements, as well as monitor potential threats in patients' external environments are a few of the many benefits of wearable biosensors[1], [2]. Measuring, maintaining are just a few of the m a n y uses biosensors have, some of these applications, particularly those intended for the sports industry, are likely to be available in medium term, which is about three to five years while, and will be made available long-term, which is above five years.

Biosensors are chemical sensors in which the analyte recognition is sensed through biochemical or biological mechanisms. The interactions between the analyte and the sensing elements determine the biological responses. In these interactions, some physical and chemical properties of the sensing elements may vary depending on the analyte concentration[3]. To assess these property changes, the sensor convert the interaction responses into measurable physical quantities. This process of analyte recognition can then be achieved through various methods, such as recognition by ions, nucleic acids, enzymes, and/or by cells and tissues. Specifically, bioreceptors are highly selective towards the analyte. **Fig. 1** illustrates the function of biosensors.

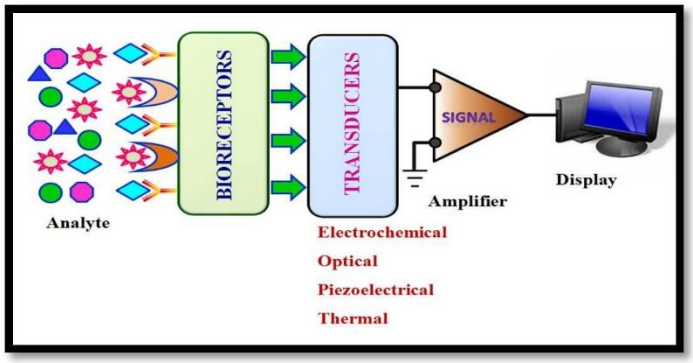


Fig. 1. Schematic representation of Function of biosensor.

1.1 Wearable biosensor:

According to Transparency Market Research, the market potential for wearable biosensors is high, and projected to reach a market value of $21.6 billion by 2020 [4]. It is projected that "The biosensors market is expected to witness considerable growth owing to its wide array of applications in diabetes monitoring, cardiac monitoring, drug discovery, agriculture, environmental and bio-defense practices"[4]. The growth of this market has been aided by the rise in the diabetic population coupled with an increase in the increased demand for home-care and point-of-care diagnostics. Innovation reduced not only the size of biosensors but reduced the pain experienced by the user as well. Primarily, the method of collecting input from the user was to prick the user to obtain a droplet of blood. To eliminate the pain of a prick but still be able to collect data, researchers came to the solution to use different media for analysis such as Interstitial Fluid (ISF), sweat, tears, saliva, and many other types of media.

The use of wearable biosensors is always beneficial as they provide continuous monitoring[5]. They are easy to use and their use reduces the hospitalization fee. Wearable biosensors are not only responsible for the measurement of a biomarker, but also sends the signals through the advanced wireless

This article has been accepted for publication in a future issue of this journal, but has not been fully edited. Content may change prior to final publication. Citation information: DOI 10.1109/RBME.2017.2706661, IEEE Reviews in Biomedical Engineering

systems to the control unit. Emergency situations are detected via data processing implemented throughout the system and an alarm message is sent to an emergency service center to provide immediate assistance to patients. This creates a two-way feedback between doctors and patients allowing patients to get advice from their doctors without physically going to the hospital. Utilizing the information provided by wearable biosensors, patients have the potential to shorten hospital stays and reduce readmissions by being informed about their health. A conceptual representation of a system for remote monitoring is shown in **Fig 2**.

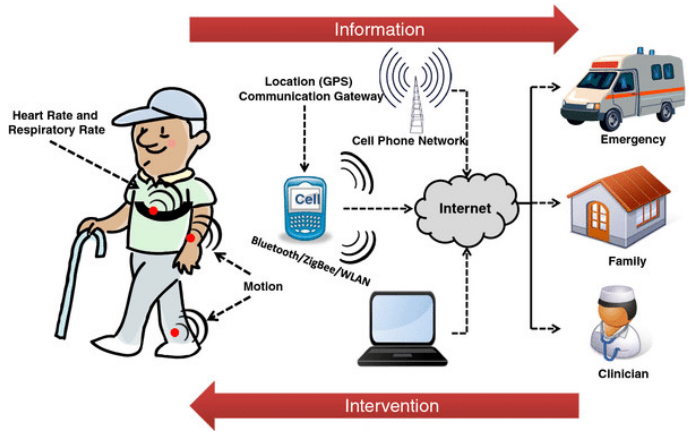


Fig.2. Illustration of a remote health monitoring system based on wearable sensors. Health related information is gathered via body-worn wireless sensors and transmitted to the caregiver via an information gateway such as a mobile phone. Caregivers can use this information to implement interventions as needed. Regenerated with the permission from [6].

There has been a growing interest in developing portable point-of-care (POC) diagnosis systems for healthcare monitoring [7]–[11]. POC technologies, which include wearable devices and biosensors, remote monitoring and real-time analysis, provide innovative solutions that promise to improve diagnosis and therapy outcomes [12]–[14]. Such POC devices can revolutionize POC medical testing and diagnosis by making testing and diagnosis fast, cheap and easily accessible. The interest for wearable systems originates from the need for real-time monitoring of patients over extensive time period. This case arises when clinician want to monitor individuals whose chronic condition includes risk of sudden acute events or individuals for whom interventions need to be assessed in the home and outdoor environment. If observations over one or two days are satisfactory, ambulatory systems can be utilized to gather physiological data. However, ambulatory systems are not suitable when monitoring has to be accomplished over periods of several weeks or months, as is desirable in a number of clinical applications. Wearable sensors can be integrated into various accessories such as garments, hats, wrist bands, socks, shoes, eyeglasses and other devices such as wristwatches, headphones and smartphone and it provides real time measurement.

1.2 Why electrochemical wearable biosensors?

An electrochemical biosensor transforms electrochemical information into an analytically useful signal[15]. The principle behind the electrochemical sensor is based on various chemical events that cause the formation of ions by changing the electrical properties of the analyte solution. Observed therefore, the electrical responses can be linked to the reactions that take place between the analyte and the biological element[15]. Electrochemical sensors are advantageous in several ways. They have also the low detection limits with small analyte volumes; they allow for easy miniaturization, robustness, and a wide range of linear response with good stability, and reproducibility.

Electrochemical enzyme-based sensors contain the high specificity of the enzyme as well as sensitivity of electrochemical transducers. The enzyme electrode carries a thin layer of immobilized enzymes on its surface[16]. A subset of electrochemical sensors is amperometric devices sensors' that continuously measure the current result from the oxidation or reduction of biochemical reactions. **Fig. 3** shows the working principle of amperometric immunosensors.

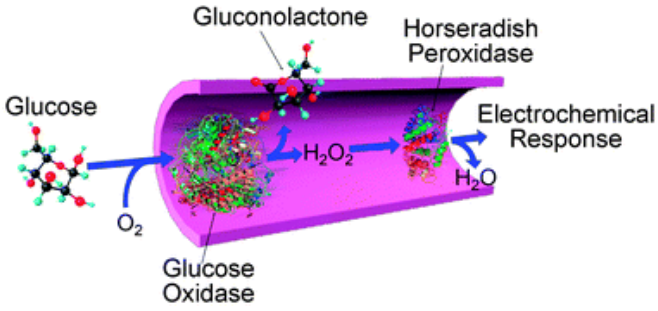


Fig. 3. An electrochemical biosensor formed by entrapping enzymes glucose oxidase and horseradish peroxidase. Adapted from [16]

There are many ways proposed to bypass the process of invasive methods to perform various tests. One of them are to have sweat samples, as sweat is a vital body fluid with relevant analytes present in it. The stability of such bio- devices and its storage time are important variables evaluate for practical purposes. The best example of this is Gamella's work[17], the stability of alcohol level detecting device from a sweat was analyzed. The bio device they proposed was to compare between currently used Breath alcohol analyzer with their proposed one. The stability they obtained is 6-8 hours for continuous monitoring[17]. To make the process of continuous monitoring more user friendly and to make it succeed in practical life, the wearable biosensors are preferred.

1.3 Stability and Shelf-life of biosensors for continuous monitoring:

In any application, stabilization is a very essential part that has to be taken into account. Stabilization of enzymes and other proteins is the main challenge when achieving viable biosensors. All products require both shelf and operational stability. Most of the achieved biological response is converted by sensors require at least 1-2 years of storage stability, known as shelf stability, whereas operational stability depends on types of biosensor. Operational stability varies from several minutes to several months[18]. The practical lifetime of the biosensor is either unknown or limited to days or weeks when they are incorporated into industrial processes or introduced to biological sample, or implanted in vivo [19]. In order to achieve a more

This article has been accepted for publication in a future issue of this journal, but has not been fully edited. Content may change prior to final publication. Citation information: DOI 10.1109/RBME.2017.2706661, IEEE Reviews in Biomedical Engineering

viable biosensor, the transducers need to be in specific state in which it generate the necessary acute biological responses. The enzyme manufacturer also needs to be consider solution stability during manufacturing process.

Enzymatic biosensors are mostly used for wearable biosensing applications, because they provide pure catalytic action during their interaction with the analyte[20]. Bioreceptors interact with the analyte to achieve biological response, which is later converted into an electrical signal by the transducer. In this interaction, the enzyme needs to be in a state in which it will generate the acute biological responses[21]. The pH and ionic strength/viscosity in the bio-fluids can vary drastically which can have an effect on the sensor's response. To counter this effect, it has recently been proposed to a recent proposition to shrink the dimensions of electrochemical sensors in order to increase the signal-to-noise ratio (SNR). Multiple enzymatic labels are used to increase the signal per event.

The enzymatic biosensors also have more specific binding capabilities and bio-catalytic activity than any other biorecognition elements such as antibodies, nucleic acids, cells and micro-organisms. The specificity of enzymatic biosensors allow them to be more efficient than cell based sensors because the diffusion paths of an enzyme are shorter due to no cell wall barriers which results in faster responses [22]. Enzymes are very sensitive to their environment; their specificity is essential for avoiding false signals. However, stability is the most important issue while working with enzymes. Enzymes can be stabilized to improve the overall shelf and operational stability of the biosensor.

Glucose biosensors use the enzyme glucose oxidase (GOx), a protein that contains the high level of glycosylation on the molecule's surface [23]. When dry, this enzyme is in stable form which allows the manufacturers to the store the device without activity loss. However, the enzyme has a level of instability when in solution or during an operation, it is not as stable. Stabilization is an essential factor to take into consideration.

II. Classification of Wearable Biosensors

2.1 On-body sensors:

Biosensors that are designed to monitor a specific parameter and that have the capability to be carried on the body are known as on-body biosensors. These are wearable sensors which make direct contact with the surface of a body part such as the skin, eye, mouth, tooth; among others, they act as basically become an extension of the human body. The most common on-body biochemical sensors are designed to perform analyses of sweat. Other less common ones analyze the tear fluid and saliva[24]. Patients benefit from these devices because of their ease of use, their undisruptive nature, and their ability to reduce the length and frequency of hospital stays.

Individuals reap these biosensors benefit, since they are easy to use, undisruptive and lead to a short hospital stays and readmissions. They come in several forms, such as including bandage, tattoo and sticker biosensors[1]. These can be in many forms and disposable and quite cost-effective. Smart on-body sensors can provide health information in an efficient and unobtrusive way. It has been observed that in many medical centers on-body biosensors are used for Point-of-Care (POC) Diagnostics. Here in this paper, the different versions of on-body biochemical sensors have been reviewed which are rising in popularity because they are innovative and user friendly. Depending on the application, there is a suitable biosensor that can serve its purpose temporarily or long term. These kinds of biosensors are used for POC diagnostics.

2.1.1 Tattoo Sensors:

Tattoo sensors are separated into two categories based upon the lifetime of the sensor: temporary and long term. Temporary tattoo sensors are designed as disposable sensors with a maximum life time of 2-3 days. Long term tattoo sensors are designed to uphold their functionality for an extended period. Here in depth we will discuss under those categorize; (i) temporary tattoos sensor and (ii) smart tattoos sensor.

a) Temporary Tattoo Sensor:

Wang[25] demonstrated that the biosensing system that of wearable temporary tattoos is capable of monitoring alcohol in a real-time and noninvasive way via the integration of printed and flexible iontophoretic-sensing electrodes with wireless electronics. The flexible tattoo-based iontophoretic alcohol monitoring patch uses transdermal delivery of a drug, pilocarpine to induce sweat via constant-current iontophoresis. followed by amperometric biosensing of the sweat's ethanol (**Fig. 4**D). The latter relies on an alcohol-oxidase (AOx) enzymatic patch along with a printed prussian blue (PB) electrode transducer. A flexible supporting module with electronic readout was also integrated for wireless data transmission (**Fig. 4**). The electrochemical performance of the alcohol biosensor was validated first in a medium buffer over a wide concentration range of 0–36 mM ethanol, which corresponded to the physiological level of ethanol found in sweat. The enzymatic sensors are selective towards ethanol and have very negligible interference with the co-existing compounds.

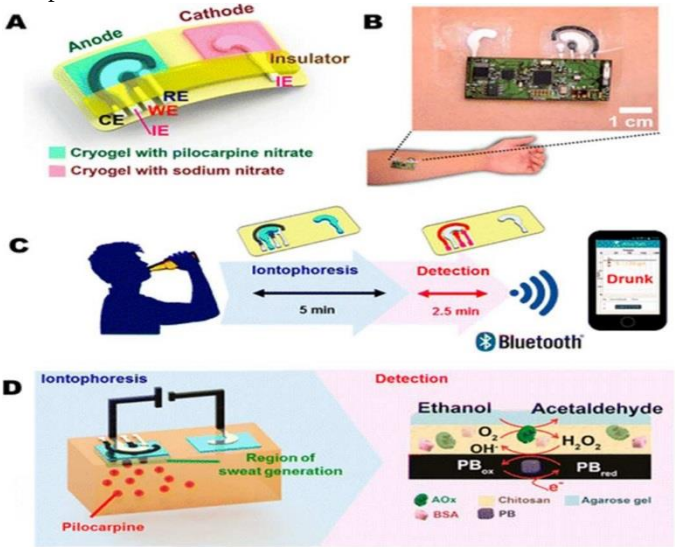


Fig. 4. Tattoo-based transdermal alcohol sensor. (A) Schematic diagram of an iontophoretic-sensing tattoo device, containing the iontophoretic electrodes

This article has been accepted for publication in a future issue of this journal, but has not been fully edited. Content may change prior to final publication. Citation information: DOI 10.1109/RBME.2017.2706661, IEEE Reviews in Biomedical Engineering

(IEs; anode and cathode), and the three sensing electrodes (working, reference, and counter electrodes: WE, RE, and CE, respectively). (B) Photograph of an alcohol iontophoretic-sensing tattoo device with integrated flexible electronics applied to a human subject. (C) Schematic diagram of a wireless operation of the iontophoretic-sensing tattoo device for transdermal alcohol sensing regenerated with the permission from:[25]

Rogers and coworkers have developed chemically sensitive wearable skin patch called "BioStamp" for sweat analysis. Chemically sensitive dye on the patch changes color as the sweat hits it, while other dyes on the patch change color in response to glucose, lactic acid, chloride, and sodium [26]. The basic Biostamp is a thin sticker about the size of an American quarter. It looks like the temporary tattoos are not reusable unlike a fully developed electronic wearable sweat monitors. Biostamp receives the biological responses by being applied directly to the skin with an adhesive which allows for it to provide more stable signal. It's a disposable sensor which sticks over the skin with a survival time of one day without shifting under it. This biostamp, the logo or symbol is printed on the body with carbon-based ink with no adverse reaction (**Fig. 5**). Some of those been further improved to be so light, durable, and comfortable enough for patients to wear them for weeks.

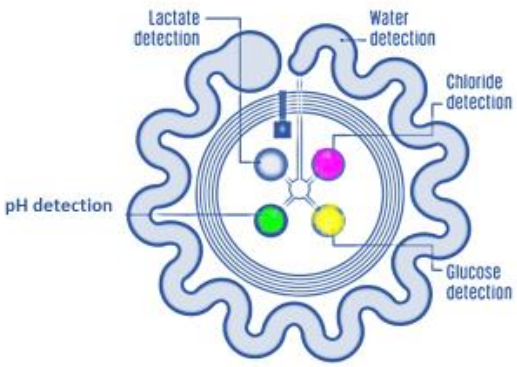


Fig. 5. Wirelessly powered Biostamp for glucose, lactate, water, and pH detection with flexible circuit with the ability which can stretch like skin. Adapted from:[27]

Wang's group have designed a noninvasive enzymatic temporary-transfer tattoo biosensor for the continuous monitoring of lactate in human perspiration [28]. It can be easily worn on body for lactate monitoring during aerobic exercise. The performance of this tattoo sensor was evaluated in terms of its selectivity for lactate measurement, the ability of adhering to epidermal surface, and the robustness when exposed to mechanical stretching and bending (**Fig. 6**A). The results have showed its potential for unobtrusive and continuous monitoring of lactate during exercise. The stability of the tattoo biosensor was examined from the response to 8 mM lactate over an 8 h period, wherein the response of the tattoo sensor was recorded every 30 min (**Fig. 6**B). The sweat pH level and lactate levels had no stability influence with enzyme activity. The high stability and integrity of the sensor was contributed by a protective coating made of mediator (tetrathiafulvalene), the BSA enzyme stabilizer, a chitosan overcoating, and the glutaraldehyde cross-linker. The shelf life of the biosensor was also examined for the ones stored at 4 °C for a period of 5 months. The responses of the biosensor remained stable during this prolonged storage period, with less than a 10% decay of the sensitivity [28].

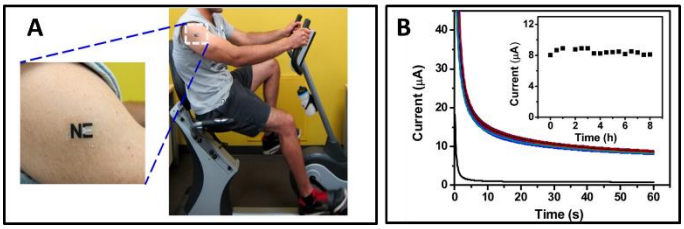
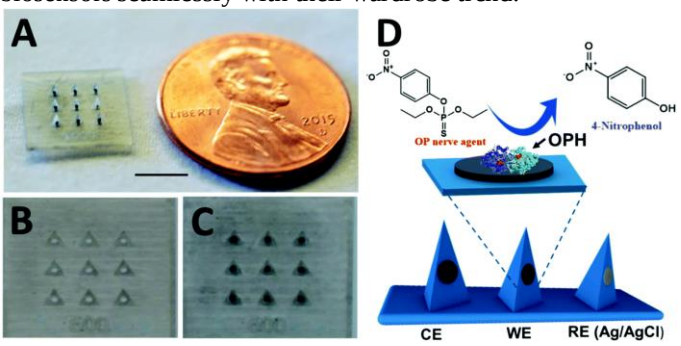


Fig. 6. A temporary tattoo lactate biosensor applied to a male volunteer's deltoid (A) and (B) Stability of the response of the tattoo biosensor to 8 mM lactate at 30 min intervals over an 8 hour period. The inset is the corresponding current−time plot of the chronoamperometric response. Regenrated with the permission from [28].

Wang's group have also explored an on-body evaluation of the tattoo-based iontophoretic-biosensing platform to detect the rise in the glucose level after a meal in a noninvasive fashion [29]. The skin-worn tattoo-based glucose detection system uses a lower current density to extract the ISF glucose followed by selective amperometric biosensing using a glucose oxidase (GOx)- modified Prussian Blue transducer at a low potential as compared to GlucoWatch. Chitosan utilized as a polymeric matrix for immobilizing the enzyme on the transducer surface while the protective layer used in the previous study[28] was used as enzyme stabilizer. In both kinds of temporary sensors, stability of an enzyme was achieved through the use of a polymetric matrix by immobilizing the enzyme on the transducer's surface[28], [29]. A microneedle electrochemical biosensor for minimally invasive detection of organophosphate has been reported by Mishra et al., [30]. This consists of enzyme-based microneedles and uses interstitial fluid (ISF) based iontophoresis techniques (**Fig. 7**). In an another report [31], Wang's team also reported microneedle based sodium sensors. The sensor data transmitted through a portable wireless Bluetooth module integrated potentiometric transducer. Nowadays, users have a variety of choices on wearing tattoo sensors. Temporary tattoo sensors are designed in many different styles with numerous logos on them. For example, University of California, San Diego has presented numerous examples of an easy to wear flexible tattoo based amperometric biosensor[25], [28], [29]. The versatility of the designs make it so that the biosensors are inconspicuous, which makes them difficult to recognize a biosensor in a tattoo. Since no one would be able to recognize that a patient's tattoo is actually a biosensor, these sensors can be not only medically functional, but also aesthetically pleasing; this encourages people to use to biosensors seamlessly with their wardrobe trend.



This article has been accepted for publication in a future issue of this journal, but has not been fully edited. Content may change prior to final publication. Citation information: DOI 10.1109/RBME.2017.2706661, IEEE Reviews in Biomedical Engineering

Fig. 7. Schematic of transdermal OPH microneedle electrode sensing. (A) Actual picture of hollow microneedle array; scale bar 10 mm. Optical images of the hollow (unpacked) (B) and carbon-paste packed (C) microneedles. (D) Schematic representation of electrochemical detection of OP nerve agents using OPH microneedle sensor based on the carbon- and Ag/AgCl-based microneedles. Regenerated with permission from:[30]

b) Smart Tattoo Sensor:

The pursuit of a more accurate blood glucose level reading led to the invention of carbon nanotube (CNT) 'tattoos'. Most recently glucose sensors involve the injection of the enzyme, GOx which breaks down glucose. These sensors have not meet the desired level of stability and are only used for seven days of use[32]. What distinguishes smart tattoo sensor is the foundation of their use of its carbon nanotubes base. In addition, these smart tattoo sensors can be immobilized in hydrogels [33],[34] that are highly biocompatible and compatible with microfabrication.

The nanotube smart tattoo sensor would change fluorescence properties in response to blood glucose, and this change could be read out using optical interrogation through the skin. This method would eliminate or reduce the need for patients to take blood samples while allowing data to be collected in a more continuous manner. Researchers used the nanoparticle inks in a saline solution that could be injected under the skin like an actual tattoo and have found that the potential lifetime of up to six months for those tattoo[35]. **Fig. 8** shows the CNT modified hydrogel based smart tattoo sensor. Glucose, lactate, and alcohol were detected using the smart tattoos.

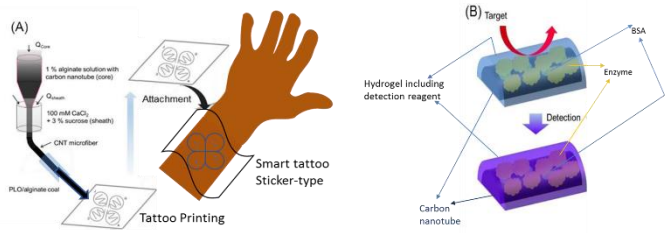


Fig. 8. A. Schematic procedure to fabricate tattoo seal biosensor. B. Schematic of detection mechanism. Each dehydrogenase and diaphorase are modified with CNT and hydrogel shells include tetrazolium for optical detection of targets. Adapted from:[32]

2.1.2 Contact Lenses Sensors:

In 2015, the Food and Drug Administration (FDA) approved Google's patent for contact lenses based sensors. These devices may help healthcare professionals to determine the optimal time of day for measuring a patient's intraocular pressure. Elevated levels of intraocular pressure associated optic nerve indicates damage that is a characteristic of glaucoma. The contact lenses are able to measure glucose and lactate concentrations [36]. The contact lenses are constructed with a tear film which consists of three layers: an outer lipid layer, aqueous layer, and the inner mucin layer. **Fig. 9** shows the pictures and configurations of the contact lenses sensors. For these types of sensors, shelf life is limited due to the degradation of enzymes that occurs because of high temperatures and exposure to light. The sensors are tested continuously for 24 hours, using 288 measurements. The stability can, however, be increased by encapsulating the enzyme [36]. Jin Zhang from a Chemical and Biochemical Engineering department, University of Western Ontario, developed the technology which uses engineered nanoparticles embedded into hydrogel lenses. The nanoparticles are engineered to react with the glucose molecules contained in tears. When sugar levels changes, a chemical reaction causes the lens to change color, allowing the wearer to adjust their glucose accordingly [37].

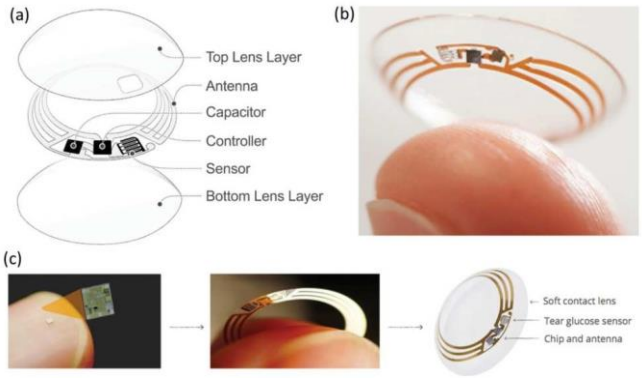
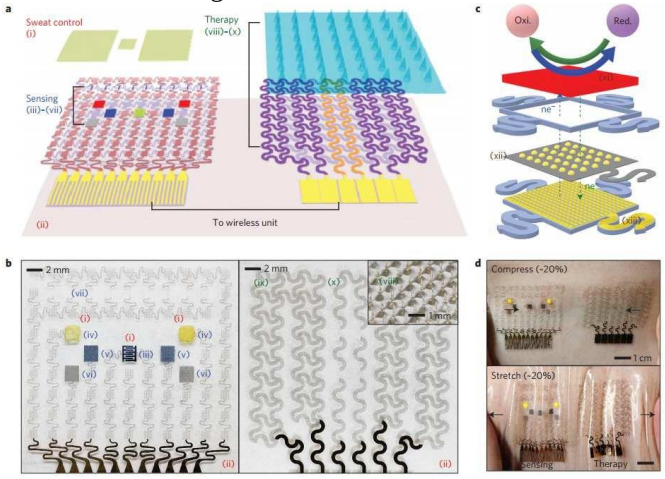


Fig 9: (a) A schematic of the contact lens sensor, showing the electrical circuitry of the sensing system. (b) The contact lens sensor prototype. (c) The wireless chip, which is mounted with the sensor, onto an electronic ring, and then embedded into the contact lens. Regenerated with the permission from: [36]

2.1.3 Patch Sensor:

A patch sensor is a flexible, transparent, skin conforming glucose monitoring device that sense glucose with a GOx enzyme interaction. They are a graphene-based electrochemical device designed by using an array of sensors patterned onto gold-doped graphene and connected by a gold mesh. Graphene is a superior material choice for its base because of its high carrier mobility, conductivity, flexibility and optical transparency.

To get accurate reading, biosensor should be activated with an enough sweat that is released from the skin. Alternatively, the patch may monitor diabetes using microneedles as well. The patch can then be connected to a portable electrochemical analyzer, which acts as a power source, and as a wireless data transmitter. When tested, the patch provided active, continuous, and stable responses under ambient conditions for several days depending of storage conditions used. **Fig. 10** provides further illustration of the patch sensor and its configuration.



This article has been accepted for publication in a future issue of this journal, but has not been fully edited. Content may change prior to final publication. Citation information: DOI 10.1109/RBME.2017.2706661, IEEE Reviews in Biomedical Engineering

Fig. 10. (a) Schematic drawings of the diabetes patch, which is composed of the sweat-control, sensing and therapy components. (b) Optical camera image of the electrochemical sensor array (left), therapeutic array (right), and magnified view of the drug-loaded microneedles. (c) Schematic of the GP-hybrid electrochemical unit, which consists of electrochemically active and soft functional materials, gold-doped graphene and a serpentine Au mesh, from top to bottom. (d) Optical camera images of the diabetes patch laminated on human skin under mechanical deformations. Regenerated with permission from:[38]

2.2 Clothing and Textile-based biosensors:

Fabric-based sensors have been used for long-term physiological signal monitoring (non-invasive or minimally invasive) because they are in constant contact with the skin. Furthermore, the large surface area of textiles provides ample space for integrating the accompanying electronics. Compared to wearable ornament systems, on-body wearable healthcare monitoring devices must be robust and durable enough to remain intact throughout tandem the wearer's daily activities. Earlier efforts in development of textile-based sensors research has led to the design of electrocardiogram (ECG), electromyography (EMG), and electroencephalography (EEG) sensing devices [15]. Recently, it was shown a bandage-based printed pH sensor used for wound monitoring [39]. Diamond's group has been active in the field of textile-based sensors and has successfully demonstrated a fabric-based conductometric sensor for measuring the extent of dehydration [20]. Other groups have also developed wearable potentiometric sensors for detection of pH, NH_{4+}, K^{+}, and Cl^{-} [40],[41]. These, have been fabricated on carbon nanotube-modified yarns using screen-printing technology with Ag/AgCl ink. Efforts have also been directed towards developing wearable sensors on flexible plastics and elastomers. Over the last few years, it has been found to be capable of monitoring some sensors a range of variables, including transcutaneous oxygen and humidity[42].

Yet, little attention, focusing primarily on biochemical sensors, has been given to wearable chemical sensors. Integration of the biochemical sensing systems contributes to the new invention of smart clothing-based sensors [1], [43], [44]. This involves integrating materials such as conducting polymers or carbon nanotubes into the fabric. **Fig. 11** will present the ideas of available styles of fabric-based biosensors.

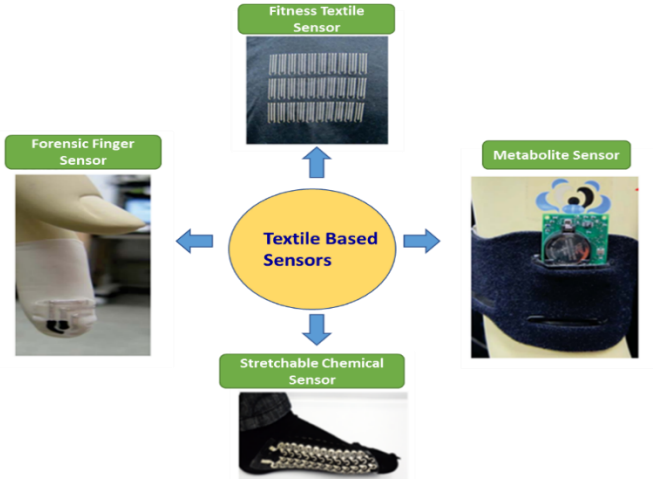


Fig. 11. Various textile biosensors, Adapted from: [47]

New fabrication methodologies and electrochemical techniques have resulted in the development of chemical sensors that can improve upon conventional physical measurements with more information [45], [46].

2.2.1 Smart Shirt:

As described by Rawal et al., [43] published in the International Journal of Scientific Research, the Smart Shirt was Georgia Tech's wearable motherboard; It consisted of optical fibers that can detect bullet wounds and special sensors that can monitor the body's vital signs. These biosensors demonstrate the amperometric detection of glucose and lactate content from sweat using organic electrochemical transistor (OECT) sensors[44]. This device uses Ionophore-based PEDOT-PSS conducting polymer with ionogel solid electrolyte. The design of the smart shirt sensor systems further illustrated in **Fig. 12**. Its lifetime is longer when compared to the other temporary tattoo- based biosensors, and it uses textile-based printing with the GOx enzyme.

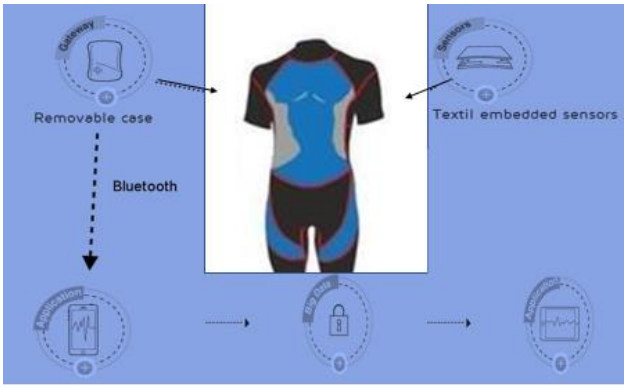


Fig. 12. Smart shirt, Adapted from: [44]

In the field of chemistry, various fabrics such as wool, cotton, and nylon are known to have a rich variety of physical and chemical properties that make them ideal for the incorporation of chemical sensors. Some textile biosensors are designed such that they have an array of printing patterns on fabrics that are easy to wash. The sources of these fabrics include animals (wool), plants (Cotton), or synthetic materials (nylon, polyester). Wearers have options to choose from in terms of structure and physical and chemical properties.

2.2.2 Cotton based Lactate Biosensors:

Saliva samples are an excellent alternative for non-invasive measurement of lactate. Cotton-based electrochemical sensors monitor lactate using the LOx enzyme with amperometry technique [48]. These are considered disposal biosensors that last longer than most others. These sensors monitor lactate continuously for 3 hours, and have been observed as capable of providing stable responses for weeks. No noticeable differences are observed when their readings are compared to the readings taken by traditional methods. The cotton fabric printed electrodes have been shown in **Fig. 13**.

This article has been accepted for publication in a future issue of this journal, but has not been fully edited. Content may change prior to final publication. Citation information: DOI 10.1109/RBME.2017.2706661, IEEE Reviews in Biomedical Engineering

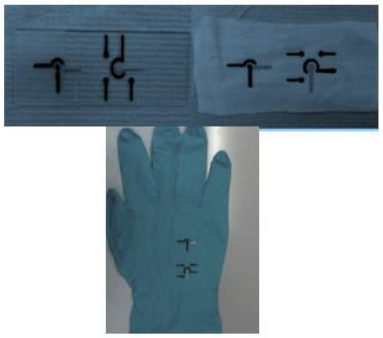


Fig. 13.: Glass microscope slide, Cotton fabric and Nitrile glove. Adapted from:[48]

2.2.3 Thick Film textile based sensor:

The textile based printed carbon electrodes usually have smooth conductor edges with no defects and cracks [49]. The Screen printed carbon electrodes on the underwear and its voltammetric scan has been shown as **Fig.14**. The favorable electrochemical behavior is maintained under fold in or stretching stress.

It is amperometric sensor which measures NADH and H_2O_2 from the body by using dehydrogenase oxide based enzyme with partial voltammetry method. This is undergarment biosensor which remains stable upon successive stretching. Direct screen printing underwear based carbon electrode is used for the operation.

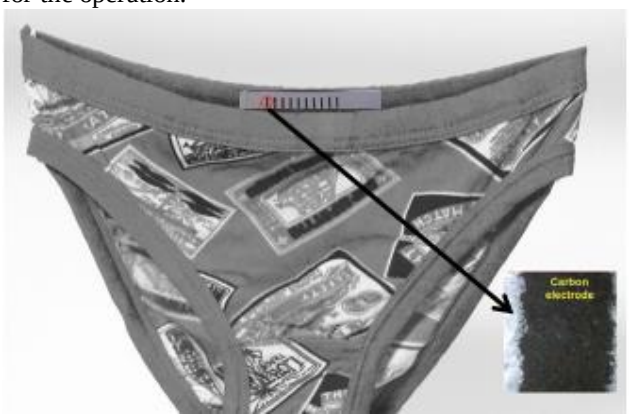


Fig. 14.: Screen –printed carbon electrodes on the underwear along with the morphology of a single electrode and linear scan voltammetric response for increasing NADH concentrations over the 0-100 uM range. Adapted from: [49]

Future applications would definitely gain the advantages from tailoring the ink composition and printing conditions as per the customer requirements.

2.2.4 Bandage Sensor:

According to the recent research[39], the smart bandage for determination of uric acid (UA) status helps a lot in monitoring the impact of chronic wounds of patients. It has become a wound healing detector, formed by screen printing an amperometric biosensor directly on a wound dressing. This amperometric sensor detects glucose with GOx enzyme, like other fabric based biosensors. The researchers have used Prussian blue carbon ink to print the electrode on the bandage as demonstrated in **Fig. 15**. The stability of the sensor has been increased by using a BSA stabilizer. It provides continuous stable responses for more than 72 hours.

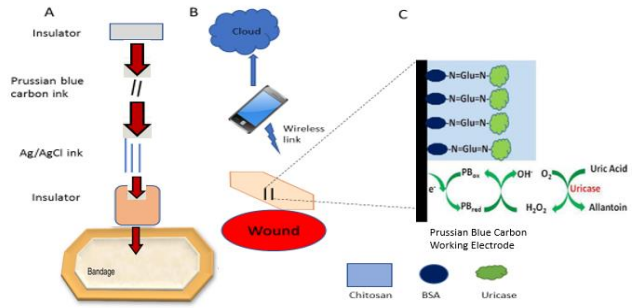


Fig 15. A. Screen printing the smart bandage B. Wearable potentiostat determines Uric Acid concentration and wirelessly communicates with a computer or smartphone. C. Schematic showing amperometric detection of Uric Acid with uricase immobilized on working electrode. Adapted from:[39]

Recent article by Kevin Mccaney [50] states that, the bandage biosensors would provide vital signs, hydration levels, stress levels and other health information by analyzing the sweat of the wearer. It has many application beyond military use, it may be helpful for athletes, firefighters or anyone whose job involves strenuous activity.

2.3 Accessories Biosensors:

Electrochemical sensors that are mobile, wearable and which loosely attach to clothing or the body classified under accessories biosensors[4]. Nowadays, wearable sensors such as Gluco Watch, pedometer, head band, arm band, wrist band, ring sensor, chip sensors are in common use. The true potential of wearable chemical sensors, which for the real-time ambulatory monitoring of bodily fluids with accessories are mentioned here. The wrist band biosensors that are used for continuous glucose monitoring comes in various forms such as wrist watch, wrist bands, and bandage. Armband sensors are used to monitor glucose with armpit odor analysis and collection of sweat. Using these techniques many factors like hydration level, pH, and biochemical markers such as lactate and glucose can be monitored and controlled [51].

While referring varieties of published articles, we came across one of the experienced manufacturing companies, Jobst Technologies GmbH and their relative technologies of making enzymatic biosensors. It has decades of experience in enzymatic biosensors [52]. According to article[53], enzyme are immobilized in a stack of four permeable polymeric membranes on top of platinum micro-electrodes. They are very thankful of the small sized of the electrodes, a single chip which can hold several electrodes. **Fig. 16** shows different kind of prototypes of accessories sensors and their interfaces[51], [54]. They used CNT/Polymer sensing layers with fabricating an array of interdigitated silver electrodes.

This article has been accepted for publication in a future issue of this journal, but has not been fully edited. Content may change prior to final publication. Citation information: DOI 10.1109/RBME.2017.2706661, IEEE Reviews in Biomedical Engineering

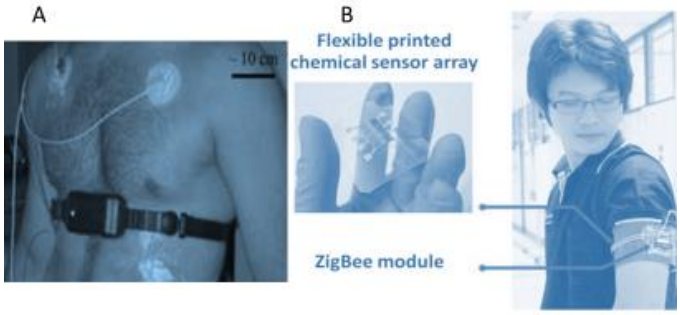


Fig. 16 A. Physiological sweat band prototype with associated electronic interfaces and the textile-based sweat pump for monitoring of human sweating. B. Armpit odour sensor fabricated from an array of interdigitated silver electrodes with CNT/polymer sensing layers, and the prototype arm band with ZigBee wireless communications. Adapted from:[51], [54]

2.3.1 Mouth guard biosensor

A concept of mouth guard metabolite biosensor has been reported by Kim et al., [55]. This is an amperometric biosensor with salivary lactate as an analyte. The direct measurement of lactate in saliva would be used as a diagnostic tool for *in vitro* monitoring as salivary lactate concentration corresponds with the blood lactate concentration. This wearable oral bio-sensory system uses LOx as an enzyme with Prussian –Blue modified electrode as transducer, acting as artificial peroxidase to offer selective detection of the H_2O_2. With the aim of stabilizing the device, LOx was immobilized on the working electrode surface by the method of polymer entrapping. It parades high selectivity, sensitivity and stability, so as to use them in getting information regarding wearer's health, performance and stress level through Bluetooth or wireless network as displayed in **Fig.17**.

Fig. 17. Mouth guard biosensor with fully integrated wireless instrumentation electronics to continuous and real time electrochemical monitoring. Adapted from:[55]

With the intention of analyzing the stability of the sensor, the researchers have taken continuous readings over the interval of 10 minutes for 2 hours and it has been noticed that the sensor displays high stability with small variations of current signal, ranging between 90% and 106% of the actual response[56]. The good stability shows the proactive actions of the Poly-orthophenylenediamine–LOx interaction, where it is used to stabilize the device. The continuous monitoring responses are hown in the **Fig.18** below:

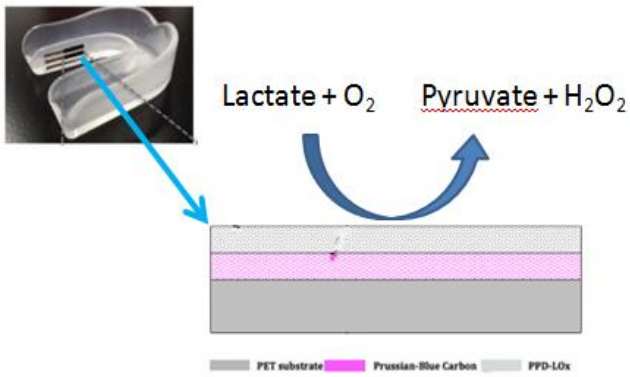


Fig. 18. Mouth guard biosensor for lactate monitoring. Adapted from:[56]

2.3.2 Wrist Watch (GlucoWatch):

GlucoWatch or GlucoWatch biographer is a wrist watch potentiostat [**Fig. 19**]. It has GOx enzyme and uses ISF to measure glucose level. This Amperometric sensor works on reverse Ionophoresis phenomenon. The readings have been taken continuously for 12-13 hours with the frequency of 3 per hour[19]. This sensor facilitates with the memory to save upto 4000 readings. It gives 78 readings per wear. After that, one has to change the sensor or stabilize the enzyme in order to continue the use. Gluco watch G2 biographer is suitable for adults and gained FDA approval for use in children and adolescents to monitor glucose continuously[19]. Patients who are insulin dependent are required to monitor their blood glucose levels to ensure that appropriate levels of insulin are circulating.

Fig. 19. GlucoWatch for continuous glucose monitoring. Adapted from:[19]

2.3.3 Wrist/Head band Biosensor

Article [57] reported a mechanically flexible and fully integrated (that is, no external analysis is needed) sensor array for multiplexed in situ perspiration analysis, which simultaneously and selectively measures sweat metabolites (such as glucose and lactate) and electrolytes (such as sodium and potassium ions), as well as the skin temperature (to calibrate the response of the sensors). These kinds of biosensors are majorly found in athlete's group for continuous health monitoring while exercising [**Fig.20**]. The device come in the form of Wrist or head band with a credit card sized amperometric biosensor embedded in it. It uses GOx and LOx enzyme which monitors glucose contents present in the sweat.

This article has been accepted for publication in a future issue of this journal, but has not been fully edited. Content may change prior to final publication. Citation information: DOI 10.1109/RBME.2017.2706661, IEEE Reviews in Biomedical Engineering

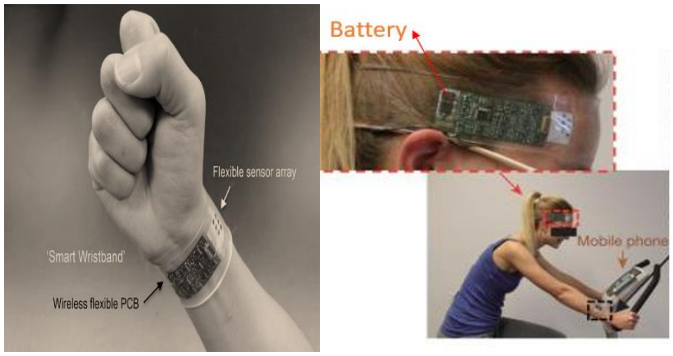


Fig. 20. Wrist and head band biosensors Adapted from:[57]

2.3.4 GCF Glucose Sensor:

To monitor blood glucose level, one method has been used where to realize a non-invasive blood glucose monitor, the Gingival Crevicular Fluid (GCF) was measured. The device to collect GCF was developed that was designed to be disposal, biocompatible and small enough to be inserted in the gingival crevice for collection of micro liters sample of GCF[58],[59]. **Fig. 21** shows working principle of GCF biosensor device and its calibration curves for Capillary Blood Glucose (CBG) and GCF [58]. It senses glucose with the help of GOx enzyme. They monitored continuous responses with increased sensitivity, accuracy, repeatability and specificity. The electrode used is ferrocene modified gold film electrode. Enzyme immobilization was done with cross-linking method. It is a saliva based noninvasive glucose monitoring tool which is widely used for clinical diagnostics. As the repeatability and ultimately stability is higher, it is used in diabetes instantaneous glucose monitoring [58].

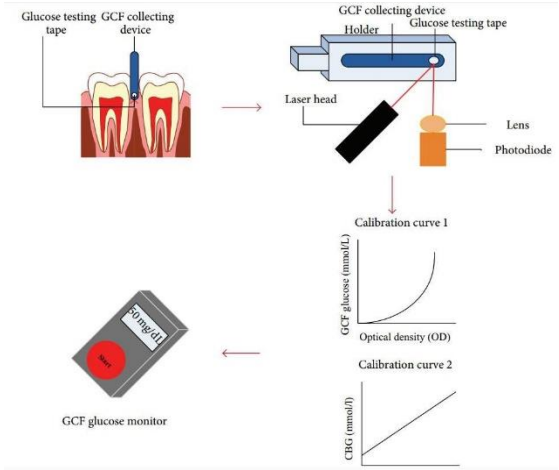


Fig. 21. Working principle of GCF biosensor for glucose monitoring with calibration curves Regenerated from:[60]

2.3.5 Packaged Lactate Chip sensor:

The electrochemical and biological interferences from saliva were discriminated by using a dual platinum electrode, common Ag/AgCl reference electrode and blocking membranes [61]. This is saliva based noninvasive biosensor which monitors lactate level in saliva. It has high operational stability and long term continuous salivary lactate monitoring is possible. The technique of enzyme probe electrode-analyte amperometric monitoring has been used in this type of sensor. The structure of packages lactate chip sensors can be studied through **Fig. 22** [61]. The reference electrode, counter electrode and cavity of working electrode has been packaged with sealing foil and pores. One of the three salivary glands, sublingual (SL) measurement with Lactate Oxide enzymatic detection has been conducted continuously with high stability [61].

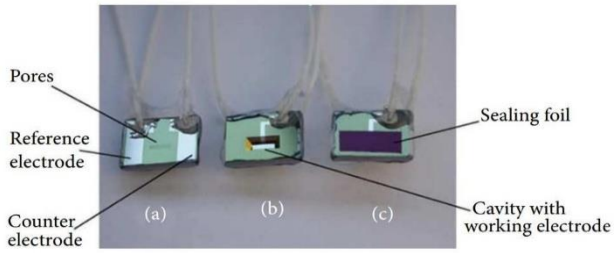


Fig. 22. Packaged Lactate Chip Sensor, Regenerated with the permission from:[61]

III. POSSIBLE ENZYME STABILIZATION METHOD:

As Enzymes react very promptly to the environment, deactivation, inhibition or unfolding upon absorption and thermal and chemical inactivation are the major causes of enzyme failure and ultimately affects the sensor results. Enzymes can deactivate in a number of different ways and some deactivation mechanisms may occur simultaneously[62]. Dehydrated enzymes are more stable than dilute solutions. Immobilized enzymes exhibit more stability than native enzymes. Encapsulation, covalent immobilization and cross-linking are the major immobilization strategies followed for designing enzymatic biosensors[63]. As compared to direct immobilization, encapsulation provides more advantages. Encapsulation retains the enzymatic activity by providing more natural environment. Also, it increases the concentration of immobilized enzymes[63]. We can encapsulate the enzymes by using polyelectrolyte multilayer capsule, vesicles, hydrogels sol-gel. Some other stabilization techniques are enzymes bound to nanoparticles or fibers and single enzyme nanoparticles. To improve operational stability, it is suggested to use large polyelectrolytes and low molecular weight electrolytes. Therefore, the lifetime of enzymatic biosensors depends on the loss of enzyme activity per time and average lifetime is around 2-8 weeks[62]. Though different techniques available to stabilize the biosensors, there are some drawbacks also associated with them. In order to overcome those, researchers are still working on the stabilization of enzymes by considering biochemical and engineering approaches. A comparison of the stability of electrochemical enzymatic biosensors is provided in Table 1.

This article has been accepted for publication in a future issue of this journal, but has not been fully edited. Content may change prior to final publication. Citation information: DOI 10.1109/RBME.2017.2706661, IEEE Reviews in Biomedical Engineering

TABLE 1. COMPARISON OF STABILITY OF ELECTROCHEMICAL ENZYMATIC BIOSENSORS

Application	Analyte	Enzyme	Method	Stability	Technique	Ref.
On-Body Sensor						
Tattoo Sensor (Temporary) 1. Biostamp 2. Printable temporary Tattoo sensor	Glucose, Lactate	GOx, LOx	Amperometry	1. More stable signal (One day survive) 2.Used Polymeric matrix for immobilizing the enzyme on transducer surface.	Carbon based ink, Enzyme based microneedle sensors (ISF), Iontophoresis electrode	[27],[29] [42]
Smart Tattoo (Sticker type tattoo)	Glucose, Lactate	GOx	Amperometry	Long useful lifetime	CNT ink for fabrication and immobilize GOx on surface of CNT. Designed in such a way that they can avoid the foreign body response of the immune system.	[21],[32], [25]
Contact Lense Sensor	Glucose, Lactate	GOx	Amperometry	Shelf life is limited. Degradation of enzyme due to light exposure and Temperature. Tested for 24 hours continuously. 288 measurements. Can be increased with encapsulating enzyme.	Electrophoretic Technique	[36]
Patch Sensor	Glucose	GOx	Amperometry	Stable for 24 hours.	Graphene based device, synthesized by CVD process.	[28], [38]
Clothing and Textile based biosensors						
Screen printed on fabric (Smart Shirt)	Glucose, Lactate	GOx	Amperometry	Stability more than tattoo based sensor.	Ionophore based ISE PEDOT-PSS for OECT with ionogels. Textile based printing. Potentiometry.	[43], [44]
Cotton based lactate biosensor (Saliva)	Lactate	LOx	Amperometry	3 hours continuous (Disposal and extended use)	Screen Printed electrode	[48],[60]
Thick film textile based amperometric sensor	NADH, Hydrogen Peroxide	Dehydrogenase oxide based enzyme	Amperometry, Voltammetry	Remains stable upon successive stretching.	Direct screen printing, Underwear based carbon electrodes	[49]
Bandage biosensor	Glucose	GOx	Amperometry	Increased stability with BSA stabilizer (over 72 hours)	Screen Printing	[39],[50]
Accessories Biosensor						
Wrist Watch (Gluco Watch)	Glucose	GOx	Amperometry	12-13hours	Reverse Iontophoresis	[19],[68]
Wrist/head band	Glucose	GOx, LOx	Amperometry	Quite stable (used in athletes)	Smart card sized potentiostat	[57],[69]
Packaged lactate chip	Lactate	LOx	Amperometry	High operational stability, Long term continuous SL monitoring possible.	Enzyme probe	[60], [61]
GCF Glucose sensor	Glucose	GOx	Amperometry	High accuracy, repeatability, specificity, Sensitivity.	Ferrocene modified film electrode	[58], [59], [60]
Mouth guard biosensor	Lactate	LOx	Amperometry	2 hours continuous operation and also eveluated long term stability.	Integrated printable 3-electrode system	[55],[56]

This article has been accepted for publication in a future issue of this journal, but has not been fully edited. Content may change prior to final publication. Citation information: DOI 10.1109/RBME.2017.2706661, IEEE Reviews in Biomedical Engineering

IV. FUTURE ASPECTS

Considering future demands of biosensors, researchers are heading towards the best possible solutions to improve the methods of stabilization and achieve the most viable wearable biosensor. In order to maintain the catalytic activity of enzyme in sensors, the sample environment is also a crucial factor to be considered. The previous stabilization strategies have failed because of the diffusion of key reactants and products in and out of the enzyme or matrix surface. For oxidase based enzymes, the coproduced hydrogen peroxide might degrade the enzyme structures [64]. Therefore, to improve the stability of GOx, some new techniques have been proposed which including cross-linking, silica sol-gel encapsulation, and molecular cloning [20]. However, these techniques also have some limitations. For examples, sol-gel encapsulation involves production of some harmful organic solvents as by-products. These are capable of destabilization of encapsulated enzymes. This leads to the decrease in catalytic activity, decrease in substrate specificity and increase enzyme inhibition. The factors which are capable to create an optimum environment for entrapped GOx stability are given by **Fig. 23**. Molecular cloning is proposed to increase the intrinsic molecular stability [20]. It helps to maintain thermal resistance and pH stability of enzymes. Near future technique to improve the stability is modification of enzymes' molecular structures. The modification of enzyme structure as per the requirements is most promising and versatile method to gain the stability without affecting performance of biosensors. Another aspect to improve the stability is to incorporate enzymes on a hydrogel or nanogel matrix. These nanogels creates protecting layers by encapsulating the bioreceptors, controls diffusion process and enhances biocompatibility.

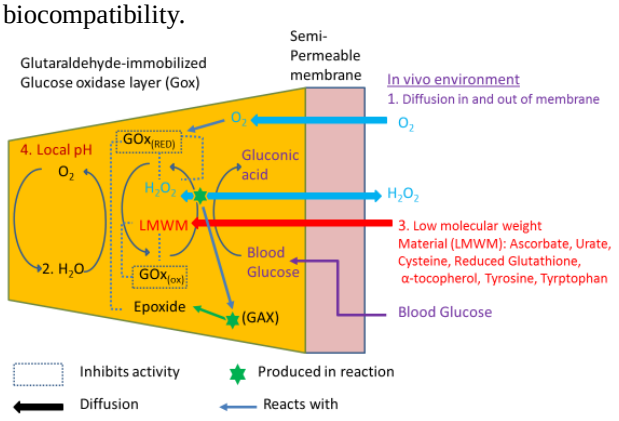


Fig.23. The factors to create an optimum environment for entrapped GOx stability: (1) ample diffusion of key reactants and products in and out of the matrix/GOx/platinum surface, (2) adequate supply of reactants and structural molecules to promote proper folding (potential barriers toward GOx stability), (3) potential degradation from LMWM and H_2O_2, and (4) loss of localized pH near the isoelectric point of GOx. Adapted from [64].

Nanohydrogels plays important roles in enhancing the biosensor performance by (i) extending the planar artificial electrode to the 3D organic matrix to increase the effective interface area, (ii) providing linkages between soft and hard materials for favorable enzyme immobilization, (iii) interfacing the ionic transporting phase and the electron transporting phase to lower the impedance, and (iv) providing high density loading with catalytic nanoparticles to promote electron collection. Also, it has been proposed that the decrease in size of substrate using nanomaterials can lead to the increase of Signal–to-Noise Ratio (SNR) and ultimately, the increase the stability [65]. There also are many different types of wearable biosensors, like sock biosensors, gloves biosensors, ring biosensors and many more [43]. Research is in process to incorporate electrochemical sensors into these platforms. The future of wearable biosensors can be painted as more durable, more stable, easy to wear, and also inclusion of various features to monitor and also, to regulate the biological element more efficiently. It will definitely help the people to take care of themselves very easily and reduce the efforts of taking doctor's appointment and waiting to be treated. This will assist doctors too, to get the continuous reports of patient's health. An exciting, future direction towards developing biosensor with long-term stability could be looking for stable artificial matrices inspired by nature to design specific materials and chemistry as an alternative to enzymes/bioreceptors[66], [67]. The main advantages of these artificial systems will make them interesting candidates for continuous monitoring applications requiring long-term stability, such as military and defense, where continuous monitoring of health and performance over extended periods of time.

V. CONCLUSION:

The development in wearable biosensors is best example of the integration of biological and engineering sciences. It includes the research of biochemical field and understanding the interaction between biological elements with the target molecule. The use of nano-transducers has been increased in separation between transducers and bioreceptors. The immobilization and stabilization strategies can be selected based on the application. For instance, while developing a sensor where durability is not an issue, (e.g. Temporary Tattoo sensors) conventional methods of enzyme stabilization like of enzyme immobilization, cross-linking can be used. For long- term sensing applications immobilization/stabilization using enzyme cloning, sol-gel techniques, hydrogel/nanogel incorporation would be a viable option. Investigating artificial receptor system that mimic the enzymatic sensing pathway is another viable approach to design biosensor for long-term stability.

Acknowledgements:
Authors acknowledge National Science Foundation (NSF), ASSIST Nanosystems ERC (EEC-1160483

REFERENCES AND FOOTNOTES

A. References

[1] S. Ajami and F. Teimouri, "Features and application of wearable biosensors in medical care.," *J. Res. Med. Sci.*, vol. 20, no. 12, pp. 1208–1215, Dec. 2015.

[2] "You wear it so well." [Online]. Available: https://theanalyticalscientist.com/issues/0314/you-wear-it-so-well/. [Accessed: 07-May-2017].

[3] F. Bănică, "What are Chemical Sensors?," in *Chemical Sensors and Biosensors*, Chichester, UK: John Wiley & Sons, Ltd, 2012, pp. 1–20.

[4] "Biosensors Market By Application (Medical Applications, Food Toxicity Detection) By Technology (Thermal Biosensors, Electrochemical Biosensors) By End-use (Home Healthcare Diagnostics, Point of Care Testing) Is Expected To Reach USD 21.17 Billion By 2020." [Online]. Available: https://www.grandviewresearch.com/press-release/global-biosensors-market. [Accessed: 24-Jan-2017].

[5] Xiao-Fei Teng, Yuan-Ting Zhang, C. C. Y. Poon, and P. Bonato, "Wearable Medical Systems for p-Health," *IEEE Rev. Biomed. Eng.*, vol. 1, pp. 62–74, 2008.

[6] S. Patel *et al.*, "A review of wearable sensors and systems with application in rehabilitation," *J. Neuroeng. Rehabil.*, vol. 9, no. 1, p. 21, 2012.

[7] A. F. D. Cruz, N. Norena, A. Kaushik, and S. Bhansali, "A low-cost miniaturized potentiostat for point-of-care diagnosis," *Biosens. Bioelectron.*, vol. 62, pp. 249–254, Dec. 2014.

[8] C. H. Ahn *et al.*, "Disposable Smart Lab on a Chip for Point-of-Care Clinical Diagnostics," *Proc. IEEE*, vol. 92, no. 1, pp. 154–173, Jan. 2004.

[9] A. Kaushik *et al.*, "Electrochemical sensing method for point-of-care cortisol detection in human immunodeficiency virus-infected patients.," *Int. J. Nanomedicine*, vol. 10, pp. 677–85, 2015.

[10] E. A. Shirtcliff, R. L. Buck, M. J. Laughlin, T. Hart, C. R. Cole, and P. D. Slowey, "Salivary cortisol results obtainable within minutes of sample collection correspond with traditional immunoassays.," *Clin. Ther.*, vol. 37, no. 3, pp. 505–14, Mar. 2015.

[11] N. T. Brannelly and A. J. Killard, "An electrochemical sensor device for measuring blood ammonia at the point of care," *Talanta*, vol. 167, pp. 296–301, May 2017.

[12] M. Tsakalakis and N. G. Bourbakis, "Health care sensor — Based systems for point of care monitoring and diagnostic applications: A brief survey," in *2014 36th Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, 2014, vol. 2014, pp. 6266–6269.

[13] P. Manickam, A. Kaushik, C. Karunakaran, and S. Bhansali, "Recent advances in cytochrome c biosensing technologies," *Biosens. Bioelectron.*, vol. 87, pp. 654–668, 2017.

[14] A. Kaushik *et al.*, "Electrochemical Biosensors for Early Stage Zika Diagnostics," *Trends Biotechnol.*, vol. 35, no. 4, pp. 308–317, Apr. 2017.

[15] S. Coyle, V. F. Curto, F. Benito-Lopez, L. Florea, and D. Diamond, "Chapter 2.1 - Wearable Bio and Chemical Sensors BT - Wearable Sensors," Oxford: Academic Press, 2014, pp. 65–83.

[16] C.-H. Lee, T.-S. Lin, and C.-Y. Mou, "Mesoporous materials for encapsulating enzymes," *Nano Today*, vol. 4, no. 2, pp. 165–179, 2009.

[17] M. Gamella *et al.*, "A novel non-invasive electrochemical biosensing device for in situ determination of the alcohol content in blood by monitoring ethanol in sweat," *Anal. Chim. Acta*, vol. 806, pp. 1–7, Jan. 2014.

[18] D. R. The Â Venot *et al.*, "Electrochemical biosensors: Recommended definitions and classification (Technical Report)," *Pure Appl. Chem*, vol. 71, no. 12, pp. 2333–2348, 1999.

[19] L. Mundy, T. L. Merlin, A. J. Braunack-Mayer, and J. E. Hiller, "GlucoWatch® G2 Biographer for the non-invasive monitoring of glucose levels.," Australia and New Zealand Horizon Scanning Network, 2004.

[20] D. Grieshaber, R. MacKenzie, J. Vörös, and E. Reimhult, "Electrochemical Biosensors - Sensor Principles and Architectures.," *Sensors (Basel).*, vol. 8, no. 3, pp. 1400–1458, Mar. 2008.

[21] A. Chaubey and B. D. Malhotra, "Mediated biosensors," *Biosens. Bioelectron.*, vol. 17, no. 6–7, pp. 441–456, Jun. 2002.

[22] P. S. Panesar, S. S. Marwaha, and H. K. Chopra, *Enzymes in food processing : fundamentals and potential applications.* New Delhi,Banglore: I K International Publish, 2010.

[23] E. W. van Hellemond, N. G. H. Leferink, D. P. H. M. Heuts, M. W. Fraaije, and W. J. H. van Berkel, "Occurrence and Biocatalytic Potential of Carbohydrate Oxidases," *Adv. Appl. Microbiol.*, vol. 60, pp. 17–54, 2006.

[24] M. Mascini, "A Brief Story of Biosensor Technology," in *Biotechnological Applications of Photosynthetic Proteins: Biochips, Biosensors and Biodevices*, Boston, MA: Springer US, 2006, pp. 4–10.

[25] J. Kim *et al.*, "Noninvasive Alcohol Monitoring Using a Wearable Tattoo-Based Iontophoretic-Biosensing System," *ACS Sensors*, vol. 1, no. 8, pp. 1011–1019, Aug. 2016.

[26] D. L. Chandler, "John Rogers and the Ultrathin Limits of Technology: His Flexible, Skin-Mounted Biostamp Is Changing the Game for Wearable Diagnostic Devices," *IEEE Pulse*, vol. 7, no. 1, pp. 9–12, Jan. 2016.

[27] T. S. Perry, "Giving your body a «check engine» light," *IEEE Spectr.*, vol. 52, no. 6, 2015.

[28] W. Jia *et al.*, "Electrochemical Tattoo Biosensors for Real-Time Noninvasive Lactate Monitoring in Human Perspiration," *Anal. Chem.*, vol. 85, no. 14, pp. 6553–6560, Jul. 2013.

[29] A. J. Bandodkar, W. Jia, C. Yardımcı, X. Wang, J. Ramirez, and J. Wang, "Tattoo-Based Noninvasive Glucose Monitoring: A Proof-of-Concept Study," *Anal. Chem.*, vol. 87, no. 1, pp. 394–398, Jan. 2015.

[30] R. K. Mishra *et al.*, "A microneedle biosensor for minimally-invasive transdermal detection of nerve agents," *Analyst*, vol. 142, no. 6, pp. 918–924, 2017.

[31] A. J. Bandodkar *et al.*, "Epidermal tattoo potentiometric sodium sensors with wireless signal transduction for continuous non-invasive sweat monitoring," *Biosens. Bioelectron.*, vol. 54, pp. 603–609, 2014.

[32] F. Ozawa and S. Takeuchi, "Sticker-Type Smart Tattoo Based on Enzyme-Modified Cnt Microfibers for Wearable Health Monitoring," *Proc. μTAS*, pp. 1580–1582, 2015.

[33] D. Kiriya *et al.*, "Meter-Long and Robust Supramolecular Strands Encapsulated in Hydrogel Jackets," *Angew. Chemie Int. Ed.*, vol. 51, no. 7, pp. 1553–1557, Feb. 2012.

[34] G. R. Hendrickson *et al.*, "Bioresponsive hydrogels for sensing applications," *Soft Matter*, vol. 5, no. 1, pp. 29–35, 2009.

[35] P. W. Barone *et al.*, "Modulation of Single-Walled Carbon Nanotube Photoluminescence by Hydrogel Swelling," *MIT News*, vol. 3, no. 12, pp. 3869–3877, 22-Dec-2009.

[36] N. M. Farandos, A. K. Yetisen, M. J. Monteiro, C. R. Lowe, and S. H. Yun, "Contact Lens Sensors in Ocular Diagnostics," *Adv. Healthc. Mater.*, vol. 4, no. 6, pp. 792–810, Apr. 2015.

[37] "Color-changing contact lenses alert diabetics to glucose-level changes." [Online]. Available: http://newatlas.com/color-changing-contact-lenses-diabetic-glucose/13682/. [Accessed: 07-May-2017].

[38] H. Lee *et al.*, "A graphene-based electrochemical device with thermoresponsive microneedles for diabetes monitoring and therapy," *Nat. Nanotechnol.*, vol. 11, no. 6, pp. 566–572, Mar. 2016.

[39] P. Kassal *et al.*, "Smart bandage with wireless connectivity for uric acid biosensing as an indicator of wound status," *Electrochem. commun.*, vol. 56, pp. 6–10, 2015.

[40] M. Parrilla, R. Cánovas, I. Jeerapan, F. J. Andrade, and J. Wang, "A Textile-Based Stretchable Multi-Ion Potentiometric Sensor," *Adv. Healthc. Mater.*, vol. 5, no. 9, pp. 996–1001, May 2016.

[41] D. Janczak, A. Peplowski, G. Wroblewski, L. Gorski, E. Zwierkowska, and M. Jakubowska, "Investigations of Printed Flexible pH Sensing Materials Based on Graphene Platelets and Submicron RuO_2 Powders," *J. Sensors*, vol. 2017, pp. 1–6, 2017.

[42] A. J. Bandodkar and J. Wang, "Non-invasive wearable electrochemical sensors: a review," *Trends Biotechnol.*, vol. 32, no. 7, pp. 363–371, Jul. 2014.

[43] V. Rawal, A. Dhamija, and S. Gupta, "Recent advancements in wearable bio-sensor applications," *Int. J. Sci. Res. Eng. Technol.*, vol. 1, no. 5, pp. 154–159, 2012.

[44] "Cityzen smart shirt tracks your health, recharges during washing." [Online]. Available: http://newatlas.com/cityzen-smart-shirt-sensing-fabric-health-monitoring/30428/. [Accessed: 24-Jan-2017].

[45] J. R. Windmiller and J. Wang, "Wearable Electrochemical Sensors and Biosensors: A Review," *Electroanalysis*, vol. 25, no. 1, pp. 29–46, Jan. 2013.

[46] A. J. Bandodkar, R. Nuñez-Flores, W. Jia, and J. Wang, "All-Printed Stretchable Electrochemical Devices," *Adv. Mater.*, vol. 27, no. 19, pp. 3060–3065, May 2015.

[47] "Joe Wang - Nanoengineering - UCSD." [Online]. Available: http://joewang.ucsd.edu/index.php?option=com_content&task=view&id=17&Itemid=35. [Accessed: 11-May-2017].

[48] R. S. P. Malon *et al.*, "Cotton fabric-based electrochemical device for lactate measurement in saliva," *Analyst*, vol. 139, no. 12, p. 3009, 2014.

[49] Y.-L. Yang *et al.*, "Thick-film textile-based amperometric sensors and biosensors," *Analyst*, vol. 135, no. 6, p. 1230, 2010.

[50] "Biosensor bandage collects vital signs, health indicators from sweat -- Defense Systems." [Online]. Available: https://defensesystems.com/articles/2014/04/18/afrl-biosensor-bandage.aspx. [Accessed: 24-Jan-2017].

[51] P. Lorwongtragool, E. Sowade, N. Watthanawisuth, R. Baumann, and T. Kerdcharoen, "A Novel Wearable Electronic Nose for Healthcare Based on Flexible Printed Chemical Sensor Array," *Sensors*, vol. 14, no. 10, pp. 19700–19712, Oct. 2014.

[52] "New Enzymatic Biosensors are Detecting Analytes with a Higher Specificity Than Before." [Online]. Available: http://www.azom.com/article.aspx?ArticleID=13852. [Accessed: 07-May-2017].

[53] A. Weltin *et al.*, "Cell culture monitoring for drug screening and cancer research: a transparent, microfluidic, multi-sensor microsystem," *Lab Chip*, vol. 14, no. 1, pp. 138–146, 2014.

[54] A. Cazalé *et al.*, "Physiological stress monitoring using sodium ion potentiometric microsensors for sweat analysis," *Sensors Actuators B Chem.*, vol. 225, pp. 1–9, 2016.

[55] J. Kim *et al.*, "Wearable salivary uric acid mouthguard biosensor with integrated wireless electronics," *Biosens. Bioelectron.*, vol. 74, pp. 1061–1068, 2015.

[56] J. Kim *et al.*, "Non-invasive mouthguard biosensor for continuous salivary monitoring of metabolites," *Analyst*, vol. 139, no. 7, p. 1632, 2014.

[57] W. Gao *et al.*, "Fully integrated wearable sensor arrays for multiplexed in situ perspiration analysis," *Nature*, vol. 529, no. 7587, pp. 509–514, Jan. 2016.

[58] M. Yamaguchi *et al.*, "Evaluation of Time-Course Changes of Gingival Crevicular Fluid Glucose Levels in Diabetics," *Biomed. Microdevices*, vol. 7, no. 1, pp. 53–58, Mar. 2005.

[59] N. Brill and B. O. Krasse, "The Passage of Tissue Fluid into the Clinically Healthy Gingival Pocket," *Acta Odontol. Scand.*, vol. 16, no. 3, pp. 233–245, Jan. 1958.

[60] R. S. P. Malon, S. Sadir, M. Balakrishnan, E. P. Córcoles, and E. P. rcoles, "Saliva-based biosensors: noninvasive monitoring tool for clinical diagnostics.," *Biomed Res. Int.*, vol. 2014, p. 962903, 2014.

[61] C. G. J. Schabmueller, D. Loppow, G. Piechotta, B. Schütze, J. Albers, and R. Hintsche, "Micromachined sensor for lactate monitoring in saliva," *Biosens. Bioelectron.*, vol. 21, no. 9, pp. 1770–1776, 2006.

[62] Tim D. Gibson, "Biosensors: The Stability Problem," *ANALUSIS*, vol. 27, pp. 630–638, 1999.

[63] K. Rathee, V. Dhull, R. Dhull, and S. Singh, "Biosensors based on electrochemical lactate detection: A comprehensive review," *Biochem. Biophys. Reports*, vol. 5, pp. 35–54, 2016.

[64] J. M. Harris, C. Reyes, and G. P. Lopez, "Common causes of glucose oxidase instability in in vivo biosensing: a brief review.," *J. Diabetes Sci. Technol.*, vol. 7, no. 4, pp. 1030–8, Jul. 2013.

[65] I. Heller, J. Männik, S. G. Lemay, and C. Dekker, "Optimizing the Signal-to-Noise Ratio for Biosensing with Carbon Nanotube Transistors," *Nano Lett.*, vol. 9, no. 1, pp. 377–382, Jan. 2009.

[66] P. Manickam, S. K. Pasha, S. A. Snipes, and S. Bhansali, "A Reusable Electrochemical Biosensor for Monitoring of Small Molecules (Cortisol) Using Molecularly Imprinted Polymers," *J. Electrochem. Soc.*, vol. 164, no. 2, pp. B54–B59, Dec. 2017.

[67] P. Manickam, F. Arizaleta, M. Gurusamy, and S. Bhansali, "Theoretical Studies of Cortisol-Imprinted Prepolymerization Mixtures: Structural Insights into Improving the Selectivity of Affinity Sensors," *J. Electrochem. Soc.*, vol. 164, no. 5, pp. B3077–B3080, Jan. 2017.

[68] "Continuous Glucose Monitoring & AGP - Abbott FreeStyle Libre Pro." [Online]. Available: http://www.freestylelibrepro.us/. [Accessed: 20-Feb-2017].

[69] M. D. Steinberg, P. Kassal, and I. M. Steinberg, "System Architectures in Wearable Electrochemical Sensors," *Electroanalysis*, vol. 28, no. 6, pp. 1149–1169, Jun. 2016.