DEGENERATIVE DISEASES EARLY STAGES BIOMARKER DETECTION USING A SILICON CARBIDE (SiC) BASED BIOSENSOR

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Degenerative diseases early stages biomarker detection using a Silicon Carbide (SiC) based biosensor.

by

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A dissertation submitted in partial fulfilment of the requirements for the degree of master in electronic engineering in the Department of Electrical and Electronic Engineering

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Authorship Declaration

I, Brayan Montero Arévalo declare that this thesis entitled “Degenerative diseases early stages biomarker detection using a SiC (SiC) based biosensor” and the work presented in this manuscript are my own. I confirm that:

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- When I have consulted the published work of others, it is always clearly attributed.
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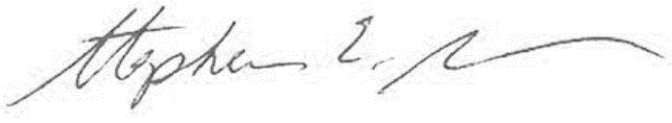
Thesis Co-Adviser Signature	

Glossary

A

Alzheimer's Disease (AD): A progressive type and cause of dementia that impairs cognitive abilities such as memory and reasoning. AD is characterized by the abnormal protein deposits accumulation of amyloid beta plaques and tau tangles in the brain.

Amino acid: A simple organic compound that contains the carboxyl (-COOH) and an amino ($-NH_2$) group. Amino acids combine to form proteins.

Amyloid Beta (Aβ): Peptides of 36 to 43 amino acids that are the amyloid plaques main component and are found in the brain of Alzheimer's disease patients. Aβ derives from the amyloid beta precursor protein (APP) and the gene precursor protein for Aβ is found in chromosome 21.

Amyloid plaques (Senile plaques): Hard and insoluble deposits of Aβ protein in the spaces between nerve cells composed of $A\beta_{42}$ fibrils.

Analyte: A chemical substance undergoing chemical analysis. In biochemistry the analyte is the substance of interest that needs to be detected e.g., amyloid beta 1-42($A\beta_{42}$) is the analyte in the biosensor for $A\beta_{42}$ detection.

Antibody (Ab): A Y-shaped protein used by the immune system to target and neutralize foreign agents. For this research, the antibody selected was the amyloid beta 1 to 28 ($A\beta_{1\text{-}28}$).

Antigen: A foreign agent, toxin or other substance which induces the immune response in the body, especially the production of antibodies. In this thesis, the antigen used was the amyloid beta 1 to 42 ($A\beta_{42}$).

Axon: A nerve fiber part of the neuron that carries electrical impulses away from the cell body to be received by other neurons.

$A\beta_{42}$ fibrils: $A\beta_{42}$ protein assemble to form insoluble fibers resistant to degradation.

B

Biomarker: A natural molecule, gene, or characteristic related to a particular pathological or normal biological process.

Biosensor: A self-contained analytical device that responds selectively to a concentration or activity of chemical species in a biological sample.

C

Carboxyl functional group: A carbon double-bonded to an oxygen and singly bonded to a hydroxyl group (-OH). An organic compound constituted of a carboxylic group is called carboxylic acid.

Cerebrospinal Fluid (CSF): An ultrafiltrate of plasma with clear and colorless appearance that bathes the central nervous system.

Crosslinking: Formation of covalent bonds that holds several polymer chains together.

Cyclic Voltammetry (CV): A electrochemical technique where the potential applied to an electrode is changed with a given rate between chosen potential limits and the current response is measured.

D

Dementia: The progressive loss of cognitive functioning, specially thinking, remembering and reasoning, to the point that interferes with daily activities.

E

Electrochemical cell: In electrochemistry is a device usually composed of a Working electrode (WE), reference electrode (RE) and counter electrode (CE) that converts chemical energy of redox reactions into electrical energy or uses it to cause chemical reactions.

Electrochemical Impedance Spectroscopy (EIS): A electrochemical technique where an oscillation voltage is applied to an electrode and the alternating current that results is measured. The system output is given in Impedance (Z).

Electrochemical stability window (ESW): The electric potential range applied to the electrode between which the substance is neither oxidized nor reduced e.g., water as electrolyte solvent has an ESW of 1.23V at 25°C. The application of metallic or carbon-based electrodes limits the potential window of an electrochemical-based biosensor to 1.23 V, at which water electrolysis occurs. However, wide energy bandgap materials, such as 4H-SiC, which has a potential window of 3.2 V, allow for the targeting of a wider range of organic molecules.

Electrolyte: A substance that releases ions when dissolved in water, resulting in a positive or negative charge upon being dissolved. The electrolytes are divided into acids, bases, and salts.

Energy band gap: The energy difference between the top of the valence band and the bottom of the conduction band of electrons. Is the minimum energy required to promote an electron from the valence band to the conduction band.

F

Functionalization: The process of adding properties or functions to a material by changing the surface chemistry of the material.

G

Glycoprotein: A molecule that consists of a carbohydrate and a protein.

H

Heme: An organic ring-shape molecule necessary to bind oxygen in the bloodstream.

I

Immunoglobulins (Ig): Or antibodies are glycoprotein molecules produced by white blood cells (plasma cells) that attach to foreign substances and assist in destroying them.

M

Molarity (M): The number of moles of solute dissolved in one liter of solution (mol/L) also called Molar concentration.

Mole (mol): The International System (SI) base unit for the amount of substance. It is defined as the amount of a chemical substance that contains as many elementary entities (atoms, molecules, ions, electrons, or photons) as there are atoms in 0.012 kilogram of carbon 12.

Monoclonal antibody (moAb or mAb): An antibody made using identical immune cell that are clones of a specific parent cell and bind a single epitope on the target protein.

Monomer: Is a molecule that can react with other molecules to form very large molecules such as polymers.

Myelinated axons: An axon surrounded by a lipid-rich material called myelin that improved neural conductivity.

N

Neurofibrillary tangles: Abnormal accumulations of tau protein collected inside neurons.

Neurofilament Light (NF-L) Chain: A protein that can be measured with immunoassays in cerebrospinal fluid and is highly expressed in large caliber myelinated axons.

O

Oligomer: A few repeating units of monomers.

Oligonucleotides: Or oligos, are short single- or double-stranded synthetic DNA or RNA used in biological applications.

P

Peptide: A molecule consisting of two or more amino acids linked in a chain, through a covalent bond, consisting in the carboxyl group of each acid being joined to the amino group of the next (-OC-NH-).

Polyclonal antibody (PAb): An antibody made using several different immune cells, with the affinity for the same antigen but bind different epitopes on the same protein.

Polymer: A large number of repeating units of monomers.

Polypeptide: A string of amino acids linked together by peptide bonds (−CO−NH−) . Polypeptides are very important because they form proteins.

S

Silicon carbide (SiC): Also known as Carborundum, is a hard dark crystalline compound of Silicon (Si) and Carbon (C). Silicon carbide is a biocompatible semiconductor very resistant to harsh environments.

T

Tau (Tubulin Associated Unit) protein: A protein that helps with the stability of the internal skeleton (axon) of neurons.

Thiol: An organic compound that contains the group -SH attached to an alky or other organic substituent.

Abstract

Diseases such as cancer, Alzheimer's and Parkinson's have some characteristics in common: they can be highly degenerative and exhibit most of their symptoms in advanced stages of the disease, stages in which treatment to reverse the disease is not possible and is only focused on improving the patient's quality of life and prognosis. As early dementia mental degeneration symptoms overlap with other conditions, a thorough differential diagnosis process must be used that most likely results in an untimely diagnosis, at a stage where steps to stop disease progression are too late. It is worrying that even when these diseases are manifested, their diagnoses include complex and expensive procedures, such as imaging based on iterative reconstruction models such as MRI (Magnetic Resonance Imaging), CAT (Computed Tomography Scans), PET (Positron Emission Tomography) and SPECT (Single Photon Emission Computed Tomography), ultrasound and ELISA (enzyme-linked immunosorbent assays), among others.

In the search for alternative diagnostic tools, the medical and scientific community have found that biomarkers, a biological indicator in the human body such as DNA, proteins, and cancer cells, can be detected using biosensors. This offers possible diagnosis or, at least, early indicators of risk associated with a biomarker. An example of promising biomarkers for detection are Beta-amyloid ($A\beta$) peptides. These are associated with Alzheimer's disease and are found in the human body naturally. However, its aggregation can lead to the formation of amyloid plaques, also called senile plaques in brain regions. Recent studies indicate that beta amyloid levels can be monitored in the blood, in cerebrospinal fluids (CSF), and even in saliva, allowing for diagnosis in the early stages of the disease.

In summary, the early detection of biomarkers could be a significant factor in the short- and long-term detection of degenerative disease in patients. Therefore, in this work, the research topic of interest was to study an early detection mechanism for $A\beta$ biomarkers, through a biosensor, that could later be scaled, modified, and used for the development of early point of care diagnostic tools that could facilitate a timely diagnosis of dementia diseases. This research validates the detection of amyloid-beta 42 ($A\beta_{42}$) using a Silicon Carbide (SiC) electrode, a fact that is unprecedented in the literature. $A\beta_{42}$ is considered a reliable biomarker for AD detection according to previous studies. In this thesis, the process to validate the detection with a SiC-based electrochemical sensor,

a gold (Au) electrode-based electrochemical sensor was used as a control. The same cleaning, functionalization and $A\beta_{1–28}$ antibody immobilization steps were used on both electrodes. Sensor validation was carried out by means of Cyclic Voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS) aiming to detect an 0.5 $\mu g \cdot mL^{-1}$ $A\beta_{42}$ concentration in 0.1 M buffer solution as a proof of concept. A repeatable peak directly related to the presence of $A\beta_{42}$ was observed, indicating that a fast SiC-based electrochemical sensor was constructed and may prove to be a useful approach for the early detection of AD.

Dedication

I dedicate this dissertation to:

To my father for being my role model and source of motivation, for his words that always help me and come to me at the most opportune moments.

To my mother for her unconditional support, her sweet words every day. Her sacrifice in leaving her career as a teacher to teach me from home and become my first teacher.

To my sister for helping me, for being there, for always being one step ahead and paving the way to make things easier for me.

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My sincerest thanks To Dr. Arash Takshi who welcomed me into his laboratory and did not hesitate to help me when I asked for it and to his wonderful group of students who became my family during my short stay, especially Bianca Seufert for her great hospitality and with whom I worked to carry out each of the tests of this investigation.

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I would also like to acknowledge the financial support provided by Dr. Stephen Saddow and Dr. Arash Takshi, at USF, who in the spirit of science and collaboration provided the materials, supplies and access to the facilities that allowed the development of this experimental work.

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1. INTRODUCTION

In the medical field of degenerative disease prevention, one of the principal objectives is early detection in order to stop or reverse disease progression, one of the tools that makes this possible is detection using biomarkers specific to a particular disease of interest, for this case, Alzheimer's Disease (AD).

Unfortunately, people who develop AD can only have their disease detected in its final stages. This is because, both early symptoms overlap with a wide array of conditions which are not only more probable, but also can be concomitant and because of the lack of early noninvasive detection techniques readily available in clinical settings. Thus, when the first undeniable symptoms of dementia appear, the disease is already late-stage, and the patient faces a low life expectancy linked to precarious living conditions. Diagnosis at this stage is still difficult, because the tools available are highly invasive and/or expensive, which is why in recent years attention has been focused on alternative detection techniques based on biomarkers such as Tau proteins or the Amyloid Beta (Aβ) amino acid chains, A$\beta_{1\text{-}40}$ and A$\beta_{1\text{-}42}$, which may be present in the initial stages of the disease where the symptoms have not yet become present.

1.1 Motivation

Silicon Carbide (SiC) is a novel semiconductor material that has shown promise for biological applications due to its unique electrical and mechanical properties and high chemical resistance [1]. Configured as an electrochemical sensor, the techniques of cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS), a SiC electrode should allow for the detection of biomarkers immobilized on its surface. The goal of this thesis research is to determine if a SiC electrochemical sensor can detect the specific amino acid Aβ_{42} in solution and thus potentially detect AD in its early stages.

Finally, this thesis is the first step of a larger research program (§Future Scopes), where factors such as detection in other bodily fluids will be contemplated. It is important to note that in early stages of AD some biomarkers are found in higher concentration in the body but absent in the brain and the CSF. Thus, if possible, employing a SiC-based biosensor in other regions of the body may allow us to develop devices for rapid and early detection of the disease, hopefully

allowing for early detection to greatly extend the lifetime and life quality for AD patients and their families.

1.2 Objectives

1.2.1 General Objective

Functionalize and test a SiC biosensor capable of detecting Beta-Amyloid in solution.

1.2.2 Specifics Objectives

- Development of a antibody functionalization protocol in novel band-gap material (4H-SiC) electrode for early detection of Alzheimer Disease.
- Validate biosensor functionalization stages through Cyclic voltammetry (CV) and electrochemical impedance spectrometry (EIS) techniques.
- Compare electrochemical response between SiC electrode and gold electrode.
- Using antibodies available in body fluids with potential for the development of less invasive diagnostic and detection tests.

1.3 Scopes

Current medical diagnosis of diseases such as Alzheimer's include tests only for its detection in advanced stages of the disease, this project aims at setting the stage for the development of devices able to detect the disease in its early stages, where the degenerative process is still developing and, in this way, mitigate its progression, improving the patient's quality of life.

The use of current knowledge coupled with the use of novel wide band-gap materials such as SiC to test Amyloid-Beta compounds has not been reported in the literature and therefore, it is expected that this work will be an important contribution to science. However, this work is the first step in assessing the feasibility of the development of such test devices. Being the first research stage on the development of such work, the scope of this research will only include:

- The test of a proof-of-concept sensor, not the development of the final point of care rapid-testing device.
- Testing will only be performed with commercial Amyloid-Beta in solution to prove the concept. No human or animal subjects or samples will be used.
- Proof of concept will not include clinical trials. Tests will be performed on sensors using Amyloid-Beta solutions in a biosafety laboratory.

1.4 Limitations

- Clinical trials will not be carried out.
- Tests will be performed only with in-vitro Amyloid-Beta in solution. No saliva will be used to test the sensor.
- Both the antibody as well as the Amyloid-Beta, buffering solutions, reacts, and other biological compounds will be obtained from the market.
- Proteins will most likely be reconstituted from lyophilized soluble powder, and due to its cost, a single type of antibody will be tested, after identifying the most appropriate option for electrode functionalization.
- The cost of biological compounds, such as reacts and solutions, as well as the cost of laboratory time will significantly affect the amount of tests performed.

1.5 Future Scopes

Although the purpose of the research conducted during this thesis has been the detection of a biomarker present in the early stages of a degenerative disease such as Alzheimer's using a SiC biosensor, a successful detection is just the first step of a bigger research program. In general, our process would have more steps, we mention below.

- Detection of an Alzheimer's biomarker using a SiC electrode.
- Achieve the necessary limit of detection (LOD) by using the SiC electrode for use in body fluids other than CSF, specifically, saliva.
- Perform tests in a solution that replicates saliva conditions.

- Perform tests on AD and control patient’s saliva.
- Develop a Point of Care Device and its respective patent.

2. DEGENERATIVE DISEASES: THE CASE OF ALZHEIMERS DISEASE

2.1 Alzheimer’s disease a worldwide problem

Dementia, an early sign of AD, is a syndrome in which cognitive deterioration occurs and usually affects the elderly [2]. Globally, it is estimated that 55 million people suffer from dementia, of which 60% are in low-and middle-income countries and, according to the world health organization, there are 10 million new cases reported per year. AD accounts for 60 to 70 percent of dementia cases and has no known cure, although early-detection does allow for treatments to slow its progression [2]. At this point, to avoid confusion, it is good to point out that dementia is different from Alzheimer's. The fact is that AD is a type and a cause of dementia because it impairs cognitive abilities such as memory and reasoning. Dementia will not always indicate AD, but it's always good to keep track of the expressed symptoms [3].

But is Alzheimer's a disease that is suffered in solitude? The facts indicate that this disease has a great impact on families and the economy. Dementia is the seventh leading cause of death and one of the major causes of disability and dependency among elders; in the United States alone, one in three elderly people dies with Alzheimer's or another type of dementia, with more deaths than breast and prostate cancer combined. And if all this were not enough, the risk of developing AD is one in ten for men and one in five for women. Gender also makes its mark in these cases, women over sixty ages have twice the risk of dementia than breast cancer, the greatest impact continues to affect women with the highest mortality and disability rates due to dementia, more than sixty percent of caregivers are women and, of these, it is estimated that about 20% were forced to leave their work due to caregiving duties for their loved ones [4].

By 2019 the disease management world costs exceeded 1.3 trillion US dollars and half of those costs were for undocumented care provided by caregivers. In addition, it is estimated that 70% of the hours dedicated to caring for people with dementia are covered by women, thus revealing the impact of the gender gap for this disease [4]; for reference, in USA by 2022 the unpaid caregiver time due to AD was estimated to be 18 billion hours with an assessed value of 339.5 billion US dollars [5]. Not only are caregivers doing unpaid work, but also both the patient and the caregiver must at some point leave the workforce; thus, the world economic impact of the disease is also considered significant, as an example for 2023 the cost of AD in the USA is projected as 345 billion US dollars. A never-ending situation, as we already mentioned that it is estimated that

there are 10 million new cases of dementia every year [2], a discouraging figure that sets off alarms and calls for the development of early detection and treatments.

2.2 Alzheimer disease development

According to [5] "the greatest risk factors for late-onset Alzheimer's are older age, genetics, and having a family history of Alzheimer's. Age is the greatest of these three risk factors. The percentage of people with Alzheimer's dementia increases dramatically with age. Five percent of people aged 65 to 74, 13.1% of people aged 75 to 84, and 33.3% of people aged 85 or older have Alzheimer's dementia". However, this disease does not appear suddenly, but has a development associated with very marked stages, which will be addressed in the following sections.

AD is a type of brain disease caused by damage to the nerve cells in the brain, and the biggest challenge with this disease is that the first set of symptoms normally do not appear during the earliest stages of the disease. Symptoms may take 20 years or more to appear and are already the reflection of advanced, irreversible brain damage. AD, when diagnosed, results in a life expectancy of 4 to 7 years [6]. One of the most accepted hypotheses, still under study, about the origin of AD, is the so-called Amyloid cascade hypothesis [7]. This hypothesis states that AD is caused by an abnormal accumulation of Aβ plaques in various areas of the brain. These plaques act as a trigger for a cascade effect that includes neuronal injury and the formation of neurofibrillary tangles via the tau protein, which in turn leads to neuronal dysfunction and cell death for patients with AD [8], [9]. The origin of AD can be traced back to Aβ peptides, which are present in two lengths of amino acids, Aβ_{40} and Aβ_{42}, containing 40 and 42 amino acids, respectively. Aβ is produced through an enzymatic action on the amyloid precursor protein (APP) by β and γ-secretase releasing the Aβ peptide, including Aβ_{40} and Aβ_{42} monomers, with the latter considered to be the pathogenic species [8], [9]. Consequently, Aβ_{42} monomers experience conformational change, assemble into Aβ_{42} oligomers and a continuous aggregation into Aβ_{42} fibrils that then accumulates in the brain as amyloid plaques. Recent studies conducted by Castellani et al. also confirms the predominance of Aβ_{42} in affected areas of AD brain tissue [9].

The different diagnostic assessments for Alzheimer's include psychological, genetic, brain imaging, and cerebrospinal fluid (CSF) tests [10]. Psychological tests are quite inaccurate and

genetic tests are not always available. Brain imaging can be expensive while CSF tests are invasive and extremely painful for the patient [11] [12]. Considering that the treatment for AD can be more effective if it starts at the early stages of the disease, a simple, reliable, and relatively inexpensive diagnostic method can be very effective. Recent studies on antibody-based biosensors, shown in Table II, point to some promising approaches for $A\beta_{42}$ detection using different materials for sensor construction and the possibility to use electrochemical techniques for sensor validation.

2.3 Biomarkers for diagnosis of AD

Being the most prevalent cause of dementia and having a high cost of diagnosis, the search for biomarkers for early AD diagnosis is a field of great interest. Currently there are three classic biomarkers, $A\beta_{42}$, total and phosphorylated fraction of Tau proteins (t-Tau and p-Tau) [13], respectively. Another biomarker that stands out is the neurofilament light chain (NFL). These biomarkers can be observed in Figure 1. where the sites of relevant effects over the neuron cell, due to the action of these proteins are located. At present, most of the research focuses around the three classic biomarkers. The use of CSF biomarkers is very limited due to the highly invasive nature of a lumbar puncture and the secondary effects that this implies. For this reason, the most recommended is the use of blood or blood plasma for diagnosis, although other bodily fluids such as saliva have also been explored in research in recent years.

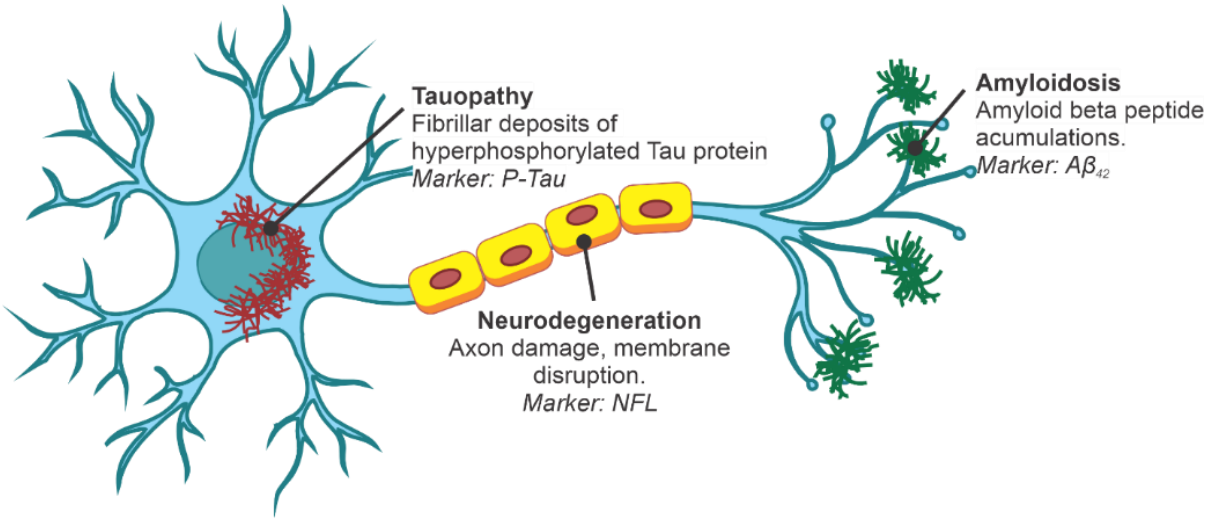


Figure 1. Location of relevant neural biological markers P-Tau, NFL and $A\beta_{42}$.

In the following subsections we will describe in detail each protein of interest.

2.3.1 Beta amyloid

Among the Aβ peptides (Aβ42, Aβ40, Aβ37, Aβ38, sAPPα and sAPPβ), the one that most correlates with physiological and pathological symptoms of AD is Aβ42. When compared with healthy controls, AD patients present low levels of $A\beta_{42}$ in the CSF [13] due to a majority of this biomarker being present in the diseased brain. As is shown in Figure 1 accumulations of Aβ peptides are usually located in axon terminals leading to senile plaques (also called amyloid plaques) found in AD patients [13].

2.3.2 Tau Protein

Two biomarkers, p-Tau and t-Tau are present in higher levels in the CSF of AD patients. More specifically, p-Tau is a property of AD and establishes a difference with other types of dementia [13] [14]. When t-Tau, p-Tau and $A\beta_{42}$ are measured together, improved specificity and sensitivity has been observed in comparison to when these biomarkers are measured alone [15] achieving an accuracy exceeding 90% [16]. Fibrillar deposits of hyperphosphorylated Tau protein (p-Tau) as neurofibrillary tangles (NFT) are present in AD patients [13].

2.3.3 Neurofilament light chain (NFL) protein

The Neurofilament light chain (NFL) is a neuronal cytoplasmic protein highly expressed in large caliber myelinated axons. Its level increases in CSF and blood proportionally to the degree of axonal damage caused by a variety of neurological disorders, including inflammatory, neurodegenerative, traumatic, and cerebrovascular diseases [11], [13].

2.4 Diagnosis of Alzheimer's disease

The diagnosis of AD is a complicated part of this disease. The lack of information tends to confuse people to the point of many clinicians not being able to distinguish between dementia and AD. To be clear AD is a cause of dementia, being that it contributes the highest percentage of patients suffering from cognitive loss.

AD is a silent disease which can only be diagnosed after the appearance of its first symptoms. These diagnoses are oriented towards the confirmation of the disease and not to its prevention. Once AD manifests itself different neuro-imaging techniques are used to identify signs that can lead to a more accurate diagnosis of AD, like Computerized Tomography (CT) Scan, Positron Emission Tomography (PET), Structural Magnetic Resonance Imaging (sMRI), Diffusion Tensor (DTI) [6]. In many instances diagnosis is only made post-mortem by dissecting the brain of the diseased patient.

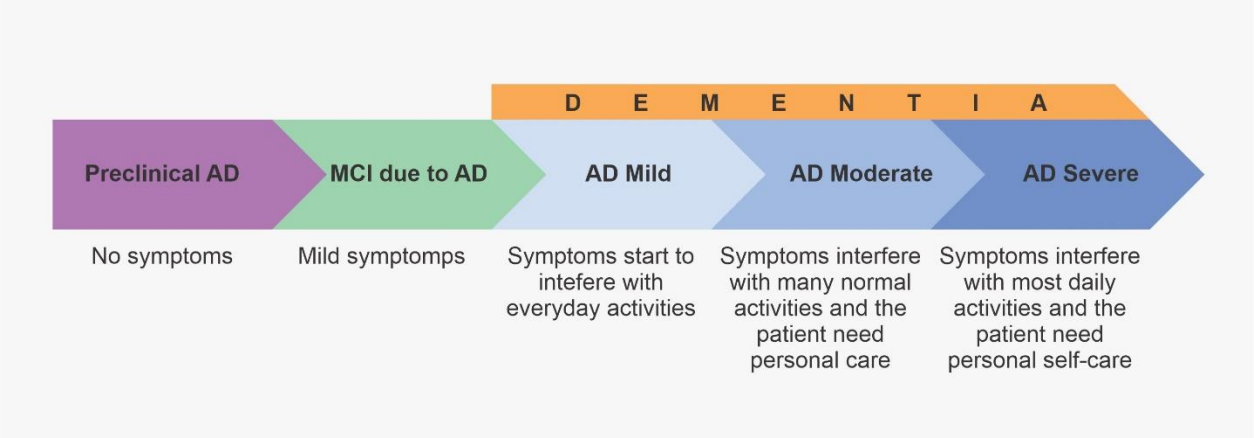


Figure 2. The Alzheimer's disease (AD) continuum.

In the field of AD diagnosis there are other alternatives to neuroimaging techniques, such as psychological tests and genetic tests which, if utilized, are performed before the afore mentioned neuroimaging techniques and offer follow-up with respect to possible development of the disease. There is a subsequent technique to neuroimaging, and it is the CSF test, which is highly invasive, painful and with potential side effects for the patient but can offer a conclusive diagnosis of the disease.

In the therapeutic approach to AD, three important fronts can be identified: Pharmaceutical via currently approved and available drugs for the treatment of the disease, which include aducanumab, donepezil, galantamine, rivastigmine, memantine, and a combination of memantine and donepezil. However, all of them have adverse effects associated; Investigational therapies that work primarily with AB and Tau pathology, including γ- and β-secretase inhibitors, α-secretase modulators, aggregation inhibitors, metal interfering drugs, medications that enhance Aβ clearance, inhibitors of tau protein hyperphosphorylation, tau protein aggregation inhibitors, and

drugs that promote the clearance of tau; Finally, alternative therapies designed to improve patient lifestyle and contribute to disease prevention [17].

With the development of Point of care devices (POC) and their positive impact on the lives of patients, a clear example of which are diabetics, the creation of biosensors for the detection of other diseases has begun to flourish. Below there is a brief explanation of how these devices work.

3. BIOSENSORS

The term biosensor is the abbreviation for biological sensor. Etymologically it could be said that it is a device that "senses life." Some authors define this term as a self-contained analytical device that responds selectively to a concentration or activity of chemical species in a biological sample [18]. We can add to this definition the one given by the International Union of Pure and Applied Chemistry, which defines a biosensor as an integrated receiver-transducer device capable of providing specific quantitative or semi-quantitative analytical information via a biological recognition element [19].

The development of a biosensor is aimed at developing a Point of Care (POC) device and its complexity is usually linked to the complexity of the fluid or medium to be analyzed [19]. The simplest biosensors are composed of 2 fundamental layers as can be seen in the Figure 3. These are the affinity or bioactive layer where the biological material, in this research the biomarkers, interact with the analyte. When interaction between biomarker and the analyte occurs, the physical properties of the biomaterial in the affinity layer change, and then the layer containing the transduction element will translate one or more of those changes into a measurable signal [19], [20].

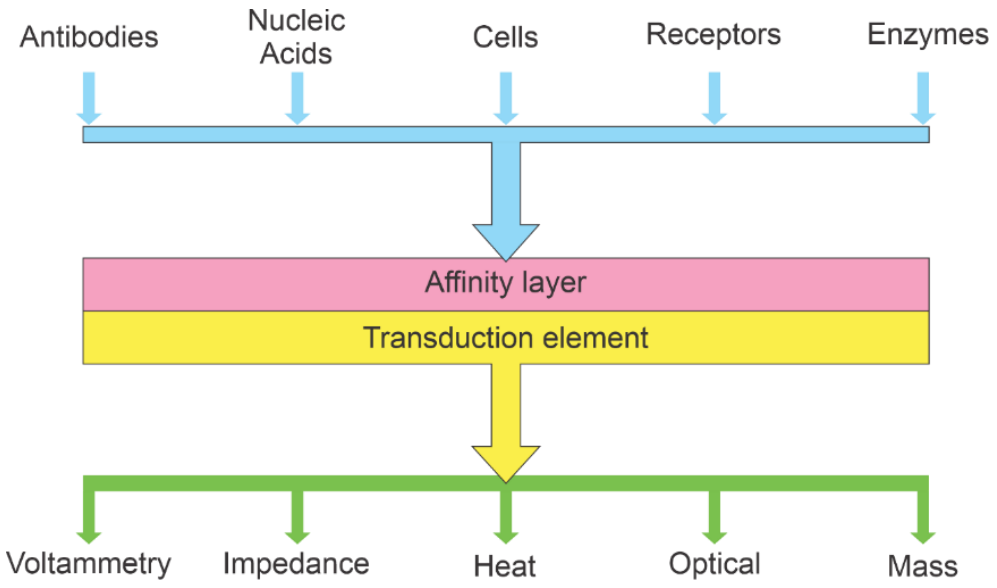


Figure 3. Simplest biosensor structure showing two primary elements: The Affinity layer that directly interacts with the biological material, and the transduction element that sends an actionable signal to the sensor output.

Now, each of these types of biomarkers mentioned earlier have been used in the development of biosensors for the detection of AD; however, one of them, the antibodies, attract the most attention due to their detection limits being close to those necessary for their detection in saliva [21]–[24]. Below we present a summary of the efforts in recent years to develop a device capable of detecting one of the accepted biomarkers of Alzheimer's, that is, $A\beta_{42}$.

3.1 Alzheimer's Disease and Biosensors

The reported studies on antibody-based biosensors are shown in Table 1, where it is observed that certain characteristics predominate, such as gold and carbon electrode materials and the use of mouse monoclonal antibodies. It is important to note that Lien et al. used a human monoclonal antibody [25], Rama et al. and Dai et al. used a rabbit monoclonal antibody [26], [27], and Li et al. and Hsu et al. used a rabbit polyclonal anti-body [28], [29]. The latter draw attention because they are more tolerant to small changes in the nature of the antigen and are reported to offer more robust detection [30]. In practice, an AD biosensor would require a liquid sample for operation, which in most cases is CSF, which requires an invasive lumbar puncture. Non-invasive attempts have been made targeting other bodily fluids, such as blood and saliva. However, in these bodily fluids, the Aβ concentration is lower than in the CSF, where the cut-off value to differentiate between patients with dementia and healthy patients is 500 $pg \cdot mL^{-1}$. This requires detection techniques that allow for a significantly lower limit of detection (LOD) [31].

As observed in Table I, most of the reported electrochemical biosensors have a LOD below the cut-off value to differentiate patients with dementia; this is due to their advantages regarding selectivity, sensitivity, and response time compared to other methods [21]. Among the parameters that can be measured with antibody-based biosensors, the following are the most common:

- Electron Current: Used in techniques such as Cyclic Voltammetry (CV), Square Wave Voltammetry (SWV), Differential Pulse Voltammetry (DPV), Chronoamperometry (CA).
- Electrical Impedance: Used in Electrochemical Impedance Spectroscopy (EIS).
- Optical Luminescence: Used in Electrochemiluminescence (ECL).

Throughout the years, electrochemical biosensors have been used for biomedical research and many medical applications, mainly due to their simplicity, affordability, point-of-care

strategies [32] and, in many cases, better LOD than other methods [21]. Additionally, antibodies are suited for element biorecognition because they provide the sensor with high specificity and sensitivity [33]. The application of metallic or carbon-based electrodes limits the potential window of an electrochemical-based biosensor to 1.23 V [19], at which water electrolysis occurs. However, wide energy bandgap materials, such as 4H-SiC, which has a potential window of 3.2 V, allow for the targeting of a wider range of organic molecules [1].

Table I. Electrochemical biosensors based on antibody for aβ42 detection.

Method[1]	**Electrode Material**	**Functionalizing Antibody**	**$A\beta_{42}$ Solution Media**[2]	**$A\beta_{42}$ Detection Range**[3]	**$A\beta_{42}$ LOD**[3]	**Refs.**	**Year**
CV	Gold electrode	$A\beta_{1\text{-}42}$	PBS	$100 - 300\ \mu M$	100 μM	[34]	2011
SWV	Carbon fiber microelectrode	mHJ2, mHJ7.4	Mice CSF	$20 - 140$ nM	20 nM	[35]	2012
CV	Gold electrode	6E10	aCSF	$0.02 - 1.50$ nM	10 pM	[36]	2012
EIS	AAO Sensing electrode	12F4	BSA	$1 - 10{,}000$ pg $\cdot$ mL^{-1}	1 pg $\cdot$ mL^{-1}	[37]	2014
CV	Screen printed carbon electrode	H31L21	BSA	$0.5 - 500$ ng $\cdot$ mL^{-1}	0.1 ng $\cdot$ mL^{-1}	[26]	2014
CV	Gold electrode	6E10 12F4	aCSF	$0.5 - 50$ nM $0.05 - 0.5$ nM	5 pM	[38]	2014
CV, EIS	Gold Electrode	6E10	Nutrient Mixture F12	-	~5 pM	[39]	2014
EIS	Carbon printed electrode	Anti-mAβ	0.02% (*v*/*v*) ammonia water at 200mM concentration	$0.01 - 100$ nM	0.57 nM	[25]	2015
CA	Screen printed carbon electrode	anti-Aβ	Human CSF, Serum and Plasma	$20 - 12500$ pg $\cdot$ mL^{-1}	19 pg $\cdot$ mL^{-1}	[40]	2015
CV, DPV	Screen printed gold electrode	$A\beta_{1\text{-}28}$	CSF	$5 - 800$ pg $\cdot$ mL^{-1}	5 pg $\cdot$ mL^{-1}	[28]	2016
SWV, EIS	Gold electrode	DE2B4	PBS	$10 - 1000$ pg $\cdot$ mL^{-1}	5.2 pg $\cdot$ mL^{-1}	[41]	2016
ECL	Glassy Carbon Electrode	anti-Aβ	PBS	80 fg $\cdot$ mL^{-1} $-$ 100 ng $\cdot$ mL^{-1}	52 fg $\cdot$ mL^{-1}	[42]	2016
SWV, CV, EIS	Glassy Carbon Electrode	anti-Aβ	PBS	$0.0001 - 100$ ng $\cdot$ mL^{-1}	0.03 pg $\cdot$ mL^{-1}	[43]	2017

[1] LOD: Limit of Detection, CV: Cyclic Voltammetry, DPV: Differential Pulse Voltammetry, LSV: Linear Sweep Voltammetry, SWV: Square Wave Voltammetry, AAO: Anodic Aluminum Oxide, mAb: Monoclonal Antibody, aCSF: Artificial Cerebrospinal Fluid, ECL: Electrochemiluminescence, ICE: Interdigitated chain-shaped electrode.

[2] BSA: Bovine Serum Albumin. PBS: Phosphate-buffered solution, CSF: Cerebrospinal fluid, aCSF: Artificial cerebrospinal fluid,

[3] The units are reported as they appear in their respective research citations.

CV	Screen printed carbon electrode	12F4, 1E11	Human Serum and Plasma	$100\ \text{fM} - 25\ \text{nM}$	100 fM	[44]	2017
DPV, EIS	Gold Electrode	EPR9296	PBS and Human Serum	$0.0675 - 0.5\ \mu\text{g mL}^{-1}$	$0.0675\ \mu\text{g} \cdot \text{mL}^{-1}$	[27]	2017
LSV	Conductive silk fibroin-based immunoparticles	mOC31	Serum	$22.5 - 1125\ \text{pg} \cdot \text{mL}^{-1}$	$3.74\ \text{pg} \cdot \text{mL}^{-1}$	[45]	2018
EIS, CV	ICE (Ti+Au)	anti-Aβ	Human Serum	$0.01 - 10{,}000\ \text{ng} \cdot \text{mL}^{-1}$	$100\ \text{pg} \cdot \text{mL}^{-1}$	[46]	2019
EIS	ICE	anti-Aβ	Human Serum	$10 - 100{,}000\ \text{pg} \cdot \text{mL}^{-1}$	$7.5\ \text{pg} \cdot \text{mL}^{-1}$	[47]	2020
DPV	Graphene-modified Screen-printed electrode	H31L21	Spiked human and mice plasmas	11 pM– 55 nM	2.398 pM	[48]	2020
EIS	Gold Electrode	12F4	PBS	$10\ \text{pg mL}^{-1} - 100\ \text{ng mL}^{-1}$	$113\ \text{fg mL}^{-1}$	[49]	2022
CV	Gold Electrode	$A\beta_{1\text{-}28}$	PBS	$0.1\ \text{pg mL}^{-1} - 10{,}000\ \text{ng mL}^{-1}$	$10.4\ \text{fg mL}^{-1}$	[29]	2022

Thanks to its wide energy bandgap, silicon carbide (SiC) is characterized by low leakage currents and very low electronic noise [50], which makes it suitable as an electrode for biosensors and neuro-implant applications [51]. Furthermore, its performance in harsh environments [52] makes it ideal for the development of reusable biosensors since it can be subjected to multiple chemical processes using etching techniques without suffering any deterioration in its chemical properties.

3.2 Alzheimer's Screening Alternatives

Aβ peptides, Tau proteins, are the main pathological biomarkers of the disease, and detection for the diagnosis of patients is progressively being implemented. However, the main problem with detection by biomarkers is that to date, its quantification is carried out mainly in CSF, whose collection requires an invasive lumbar puncture. Because of this, the diagnosis is frequently delayed until the advanced stage where the disease has already progressed. For this reason, the presence of AD indicators in other body fluids such as blood or saliva has been sought, where they could be collected in a less invasive manner [31].

To focus on the main issue, the three main fluids where detection has been made with a view to clinical applications are CSF, Blood (Plasma/Serum) and Saliva. CSF concentrates the largest amount of research pointing to three core biomarkers such as (Tau, p-Tau and Aβ_{42}) an example of this is Table I. In the case of blood, there are greater difficulties because Aβ_{42} is typically 100 times lower in blood compared to CSF, in addition plasma AB peptides are difficult to quantify because they tend to bind to erythrocytes and other plasma proteins, due to its hydrophobic nature. Finally, we have saliva, this is a very attractive fluid for detection since it is not invasive, allows repetitive measurements and it is considered that the levels of biomarkers in saliva can reflect the changes that occur in the CSF [31][53]. The following section expands a little on the investigations done in this regard.

3.2.1 Detection in Saliva

Recent studies have shown the presence of Aβ peptides in human saliva [24], [54] which opens the possibility for the development of a minimally invasive, painless, and rapid test [55] compared to those using blood and CSF (Cerebral Spinal Fluid).

In saliva, Aβ is present in small amounts, on the order of picograms. Research has shown that only Aβ_{42} peptides change significantly in patients with early and moderate Alzheimer's [23], while remaining considerably stable for advanced stage Alzheimer's patients; this is explained because in AD patients the concentration of Aβ_{42} in CSF is under 500 pg/mL which indicates that Aβ_{42} accumulates over time in the brain and as its concentration increases in the brain, less peptides are left circulating in the cerebral spinal fluid and other bodily fluids such as saliva [12]. For the detection of Aβ peptides, as a biomarker of Alzheimer's present in saliva, several tests have been carried out [22], [53] , these are better summarized in Table II.

The results in Table II demonstrate that Aβ detection in saliva is possible via mainly immunoassay conventional methods, but the use of biosensors for the same process still requires further exploration.

Additionally, the state-of-the-art shows that the detection of Aβ protein using biosensors includes different methods [21], i.e., biosensors without a recognition element, based on antibodies, on peptides, on gelsolin, on heme and finally on multiple recognition elements. To date, it is

considered that the method based in antibodies offers the highest sensitivity and requires a level of Aβ concentration that is sufficient for its detection in saliva, with a LOD that is comparable with ELISA tests [21]; this consideration makes the electrochemical antibody-based detection technique one of the most viable options, considering that the levels of Aβ_{42} in saliva are in the range of 7 to 55 pg/ml according to the references shown in Table II.

Table II. Aß$_{42}$ Detection in saliva

Type of molecule used to target Aβ_{42}	**Method used to analyze biomarkers**	**Number of subjects[4]**	**Age of the subjects[3]**	**Sex of the subjects[3]**	**Result**	**Ref.**
Rabbit polyclonal antibody (NBP2-44113)	ELISA	AD and pre-AD: 10 HC: 26 PD: 1	AD and pre-AD: 70.1 HC + PD: 54.6	AD and pre-AD: 3 M/ 7 F HC + PD:18 M/ 9 F	Aβ_{42} higher in AD patients	[56]
Aβ_{42} antibody	ELISA	AD: 15 HC: 7	AD: 77.8±1.8 HC: 60.4±4.7	AD: 7 M/ 8 F HC: 2 M/ 5 F	Aβ_{42} higher in AD patients (51.7 ± 1.6 pg/ml)	[54]
Rabbit polyclonal anti- antibody	ELISA	AD: 23 Low Controls: 25 High Controls: 6	AD: 71.3 Low Controls: 54.2 High Controls: 69	AD: 8 M/ 15 F Low Controls: 17 M/8 F High Controls: 3 M/ 3 F	Aβ_{42} higher in AD (53.95 ± 2.24 pg/ml) and High Controls patients	[57]
Drosophila cells	ELISA	AD: 20 HC: 20 PD: 20	AD: 72.5±7.68 HC: 66.1±7.79 PD: 73±8.07	AD: 8 M/ 12F HC: 9 M/ 11F PD: 5M/15 F	Aβ_{42} not detected	[58]
Monoclonal Antibody 6E10	Immunoassay with nanobeads	AD: 28 HC: 17	N.A.	N.A.	Aβ_{42} higher in AD patients	[59]
Mouse monoclonal anti-gelsolin, rabbit polyclonal anti-TTR, goat anti-mouse HRP-conjugated and goat anti-rabbit HRP-conjugated	ELISA	AD: 70 (mild: 29, moderate: 24, severe: 17) HC: 56 PD: 51	AD: 77.2 HC: 74.35 PD: 72.96	AD: 21 M/ 49 F HC: 17 M/ 39 F PD: 26 M/ 25 F	Aβ_{42} higher in mild (7.67± 16.25 pg/ml) and moderate (11.70± 34.76 pg/ml) AD patients Sensitivity and specificity around 90-95 %	[23]
Mouse anti- Aβ antibody (clone 10H3)	Mass Spectrometry	AD: 21 HC: 38	AD: 68.8 HC: 69.0	AD: 10 M/ 11F HC: 19 M/ 19F	Aβ_{42} not detected	[60]

[4] *AD: Alzheimer's Disease patients; HC: Healthy Control Patients; PD: Parkinson Disease Patients*

Studies carried out for the detection of Aβ [21] using an electrochemical sensor based on antibodies that meet the required LOD for a possible detection in saliva and that using the appropriate antibody for the detection of Aβ_{42} are shown in Table III.

Table III. Biosensors with the LOD required for detection of Aβ42 in Saliva.

Method[5]	**Electrode Material**	**Functionalizing Antibody**	**$A\beta_{42}$ Solution Media**	**$A\beta_{42}$ Detection Range**[6]	**$A\beta_{42}$ LOD**[2]	**Refs.**	**Year**
EIS	AAO Sensing electrode	12F4	BSA	$1 - 10{,}000$ pg $\cdot$ mL^{-1}	1 pg $\cdot$ mL^{-1}	[37]	2014
CV, DPV	Screen printed gold electrode	$A\beta_{1\text{-}28}$	CSF	$5 - 800$ pg $\cdot$ mL^{-1}	5 pg $\cdot$ mL^{-1}	[28]	2016
SWV, EIS	Gold electrode	DE2B4	PBS	$10 - 1000$ pg $\cdot$ mL^{-1}	5.2 pg $\cdot$ mL^{-1}	[41]	2016
ECL	Glassy Carbon Electrode	anti-Aβ	PBS	80 fg $\cdot$ mL^{-1} $-$ 100 ng $\cdot$ mL^{-1}	52 fg $\cdot$ mL^{-1}	[42]	2016
EIS	Gold Electrode	12F4	PBS	10 pg mL^{-1} $-$ 100 ng mL^{-1}	113 fg mL^{-1}	[49]	2022
CV	Gold Electrode	$A\beta_{1\text{-}28}$	PBS	0.1 pg mL^{-1} $-$ 10,000 ng mL^{-1}	10.4 fg mL^{-1}	[29]	2022

CV: Cyclic Voltammetry, DPV: Differential Pulse Voltammetry, EIS: Electrical Impedance Spectroscopy, SWV: Square Wave Voltammetry. ECL: Electrochemiluminescence Limit of detection.

The facts, up to this point, are the following: 1) in the early stages of AD, $A\beta_{42}$ is present in body fluids other than blood, such as saliva, and 2) Antibody functionalization has been successfully used to develop biosensors able to detect $A\beta_{42}$. In this thesis we propose that by combining those two statements, leads as to proposing the development of a less invasive detection point of care device, that can be used in clinical settings as a promising future solution, that to be best of our knowledge has not been reported, in any stage of development, in the literature. Such development would be an innovation of ambitious proportions; therefore, it must be tackled in stages where we can pave the ground for the design of such technology. The initial stages of development can be summarized in two necessary steps:

- Antibody selection: We made some considerations which led to determine the most appropriate antibody for this research. First, the measurement method had to be able to replicate in our laboratory. Based on this limitation, T. C. Liu et al. [42] was eliminated,

[5] LOD: Limit of Detection, CV: Cyclic Voltammetry, DPV: Differential Pulse Voltammetry, LSV: Linear Sweep Voltammetry, SWV: Square Wave Voltammetry, AAO: Anodic Aluminum Oxide, mAb: Monoclonal Antibody, aCSF: Artificial Cerebrospinal Fluid, ECL: Electrochemiluminescence, ICE: Interdigitated chain-shaped electrode. BSA: Bovine Serum Albumin.

[6] The units are reported as they appear in their respective research citations.

leaving as candidates 12F4, $A\beta_{1\text{-}28}$ and DE2B4 antibodies. After a literature review, the selection pointed to $A\beta_{1\text{-}28}$ [30] as the best Rabbit polyclonal antibody due to its better performance.

- Development of protocols: After selecting the antibody, we focused on the development of protocols for the surface functionalization of biosensor's electrodes using the aforementioned antibody for the detection of the AD marker of interest, in this case $A\beta_{42}$.

In the following sections, the processes considered to achieve the protocols are explained, beginning with the way to bind the antibody to the surface of our electrodes and finishing with the methodology for the detection of the amount of antigen that this antibody is capable of capturing.

3.3 Antibody Binding

Antibodies (Abs) and other Immunoglobulins (Igs) are highly soluble serum glycoproteins used by the immune system as a defense mechanism against agents such as pathogenic bacteria and viruses. One of its characteristics is the high specificity which allows one to recognize a unique molecule of the foreign agent, namely an antigen. They can be divided into five classes according to their heavy chain constant region sequences: IgM, IgD, IgG, IgE and IgA.

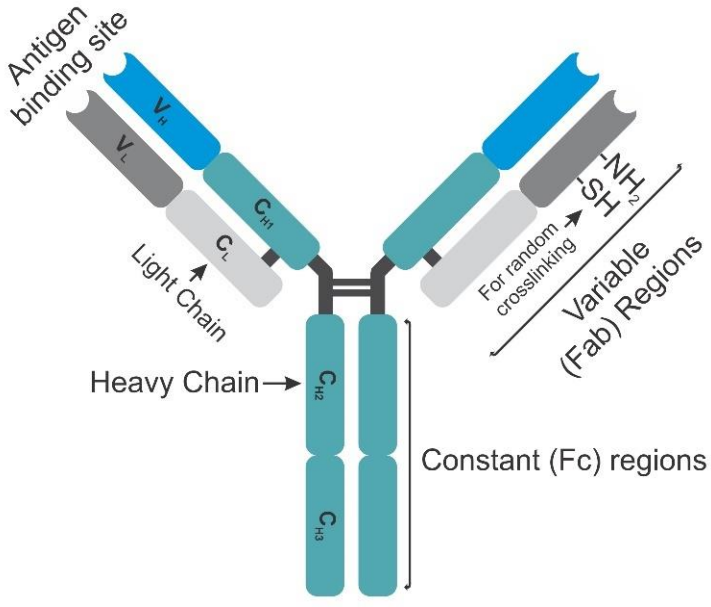
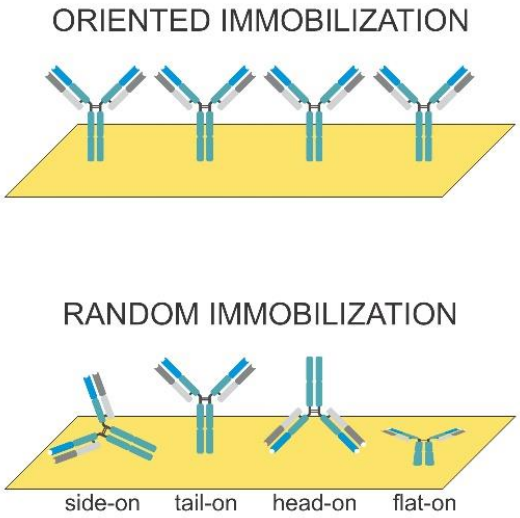


Figure 4. Structure of an IgG antibody

The basic structure of an antibody is composed of two different blocks, the antigen-binding fragment (Fab) and the constant fragment (Fc), as is seen in Figure 4. An Ab contains four polypeptide chains, two heavy and two light (either κ (kappa) or λ (lambda)). Both chains include constants (C_L, C_H) and variable domains (V_L,V_H). In addition, the specificity of the antigen binding site is determined by the variable domains of both chains and the heavy chains constant region determines the antibody class of the antibody. The hinge region connects the two heavy chains as illustrated in the Figure 4 [33], [61].

Information of interest on the orientation of the antibody on the surface is provided in Table V. An immobilized IgG can adopt 4 molecular orientations: side-on (one Fc and one Fab attached to the surface), tail-on (Fc attached to the surface), head-on (both Fabs attached to the surface) or flat-on (all three fragments attached to the surface). As is evident, for the purpose of having greater analyte binding, the antibody should display free antigen-binding regions after immobilization. In other words, if the orientation of the antibody is controlled, it will lead to a better binding of the analyte and consequently a binding improvement of the antibody resulting in a greater sensor sensitivity.

Table IV. Antibody immobilization

Immobilization Description	Type of immobilization	Type of interaction	Orientation	Functional group
	Absorption (Non-Covalent)	Electrostatic	Random	Various
		Hydrophobic Interactions	Random	
	Entrapment (Non-Covalent)	Entrapment	Random	
	Covalent Coupling	Amine coupling	Random	-NH_2
		Thiol Coupling	Specific	-SH
		Coupling via glycan moiety	Specific	-CHO
	Affinity	Intermediate protein (A/G)	Specific	Fc region
		Avidin-biotin	Specific	

In general, despite the mentioned strategies, the antibody orientation is dependent on the self-organization capacity of the antibody. Fortunately, it can be steered by specific reactive groups on the surface, on the antibody or on both. Even so, the specific orientation of an immobilized antibody is not easy to achieve.

3.3.1 Covalent Coupling

3-mercaptopropionic acid (3-MPA or MPA) and 11-mercaptoundecanoic acid (11-MUA or MUA) are sulfur containing compounds (see Figure 5), that are known to be suitable for the preparation of surface's biosensor because their bio-functional molecules, which contain both thiol and carboxylic acid groups, serve as binding sites for covalent attachment. Now, MPA and MUA offer different advantages, in the case of MUA, it allows better coverage and less defects on the surface. On the other side, MPA allows lower resistance and better selectivity [62].

It is expected that the resistance values get increased after the modification with MPA or MUA of the bare electrode surface, but regarding MPA the resistance will be lower compared to MUA. [62], [63].

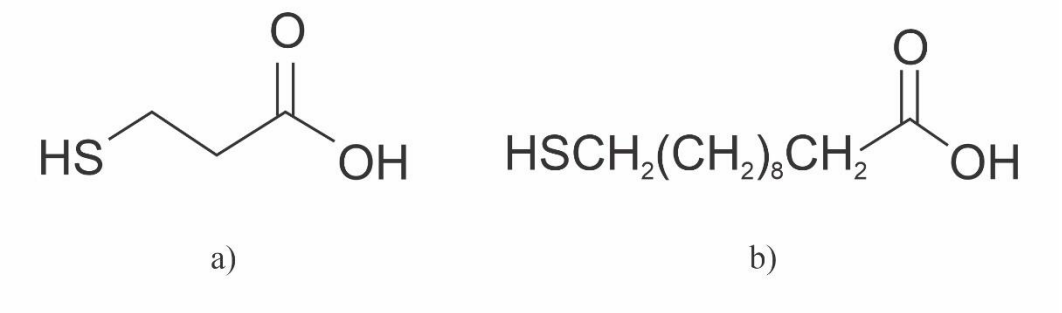


Figure 5. a) 3-mercaptopropionic acid (3-MPA or MPA) and b) 11-mercaptoundecanoic acid (11-MUA or MUA)

3.3.2 EDC/NHS Binding Technique

1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) see Figure 6.a is the most common crosslinker used in biochemical conjugations, such as two molecules of proteins, proteins and peptides, proteins and oligonucleotides, and proteins with small molecules. Nowadays, it is a common practice to use EDC with N-hydroxysuccinimide (NHS) see Figure 6.b

or Sulfo-NHS (NHS water-soluble analog) to activate carboxyl functional groups to form amine-reactive intermediates that spontaneously reacts with primary amines to form amide bonds [64] [65]. In general, NHS is used to improve the performance of EDC bioconjugation and to stabilize the amine-reactive intermediate (O-acylisourea).

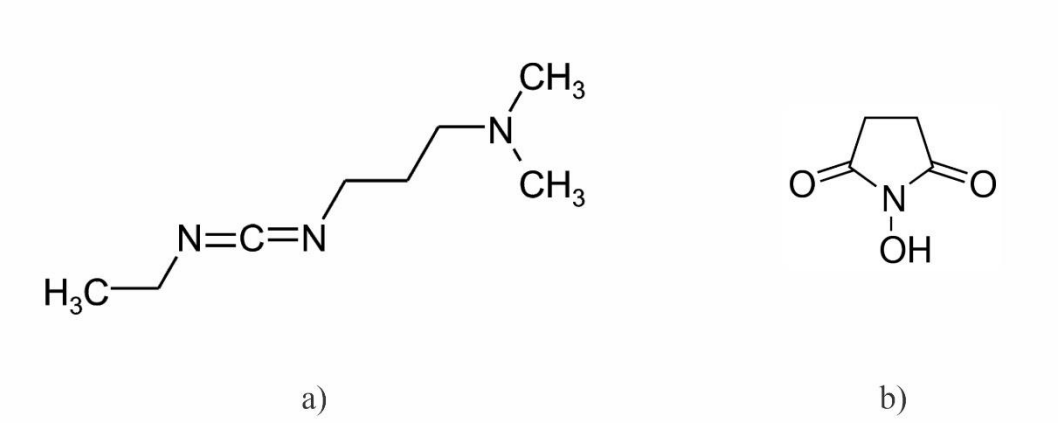


Figure 6. a) EDC and b) NHS structure

Now, regarding our research, a covalent coupling to 3-mercaptopropionic acid (MPA) via the EDC/NHS reaction has been chosen due to its proven performance with gold electrodes [26], [44], classical protein chemistry and previous reports of successful performance with SiC [51], [66]. In addition, the information presented above supports the use of this immobilization strategy.

4. MATERIALS

4.1 Silicon Carbide (SiC)

SiC is a semiconductor material with great potential to revolutionize the field of implantable devices in medicine, mainly due to its excellent biocompatibility. Among the advantages and properties of SiC are its higher power densities and lower energy losses in SiC electronic devices, it is chemically inert, mechanically resilient, possess a wide electron energy band gap, and SiC doping can be performed using many of the techniques that are performed to make Si-based devices.

It is the physical and chemical properties of this material that make it so robust and ideal for use in severely harsh environments. One example to support this statement is that the only wet chemical etchant for SiC is molten potassium hydroxide (KOH) at 400-600°C. Devices fabricated from SiC are biocompatible, electronically functional and the body does not recognize and reject them as foreign mater, which allows for their long-term use as permanently implanted devices such as glucose sensors, brain-machine interfaces, smart bone and organ implants [1].

SiC, also known as carborundum, consists of Si and C atoms that are tetrahedrally oriented, with very short bond lengths and, as a direct consequence, a strong bond strength [67], [68].

4.1.1 SiC Crystallography

SiC occurs in different crystal structures and exists over 200 different monocrystalline crystal forms called polytypes [68], [69], SiC can form amorphous, monocrystalline and polycrystalline solid forms, and the tetrahedral molecular structure allows atomic stacking in multiple directions. Despite there being so many polytypes only a few can be processed for use as an electronic semiconductor, the most common ones that have been developed for this purpose are cubic 3C-SiC, hexagonal 4H-SiC and 6H-SiC.

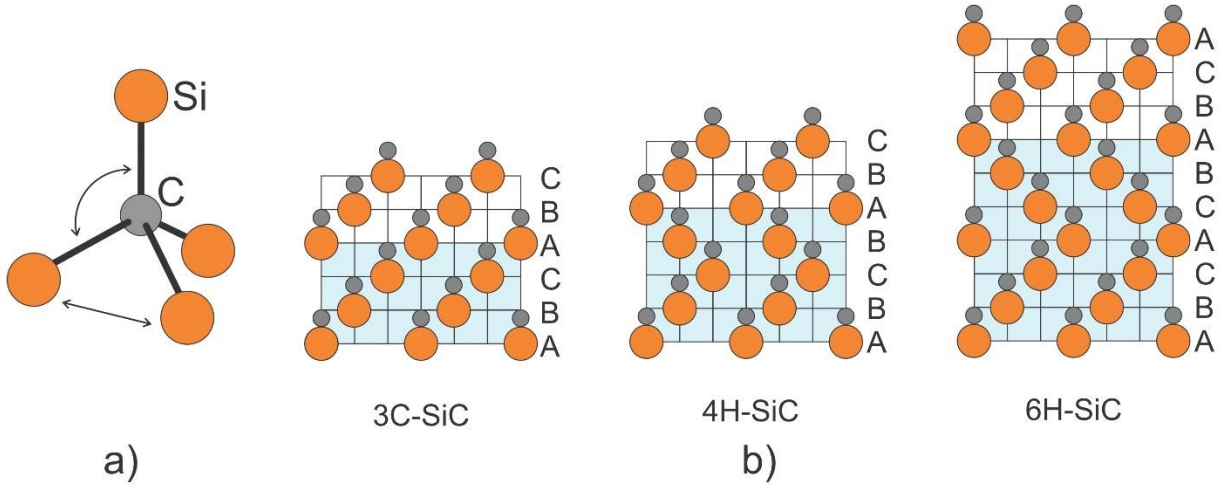


Figure 7. a) Crystal structure of SiC and b) Stacking sequence of polytypes with Ramsdell notation, from left to right, cubic (3C-SiC o β-SiC) and hexagonal (4H-SiC and 6H-SiC). A, B, and C represent the occupied sites in a hexagonal close-packed bilayer of Si and C.

For the directions and planes in the crystal, there are two cases to consider: for the cubic crystal the Miller indices are used and for hexagonal structures a1, a2, a3, and c are commonly used, as shown in the Figure 7 and Figure 8.

The SiC polytypes have an associated stacking sequence, AB (2H-SiC), ABC (3C-SiC), ABCB (4H-SiC), ABCACB (6H-SiC) and ABCACBCABACABCB (15R-SiC), changing the stacking sequence has a great effect on the electrical properties of the material, for example, from 4H-SiC to 3C-SiC the bandgap goes from 3.33 eV to 2.39 eV respectively [52].

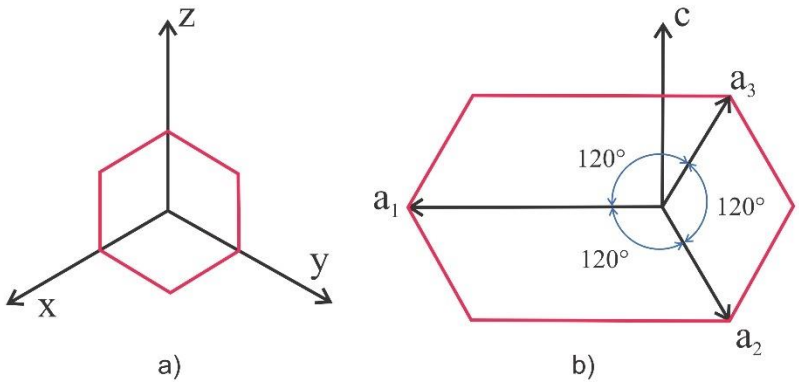


Figure 8. Principal axes for a) cubic and b) hexagonal crystals

4.1.2 Physical Properties

Within a SiC crystal the Si and C atoms form very strong tetrahedral covalent bonds, the resulting properties of SiC make a wide variety of applications possible. In general, SiC is suitable for wide variety of applications mainly due to its bandwidth gap, good electrical conductivity, excellent thermal stability and conductivity [70].

Table V. Select physical properties of the 3C, 4H and 6H polytypes of SiC at room temperature.

Properties/polytype	3C-SiC	4H-SiC	6H-SiC
Lattice parameters (Å)	a = 4.3596	a = 3.0798 c = 10.0820	a = 3.0805 c = 15.1151
Density (g cm $^{-3}$)	3.21	3.21	3.21
Young's Modulus (GPa)	350-650	350-650	350-650
Poisson's ratio	0.21	0.21	0.21
Specific heat capacity (J g $^{-1}$ K $^{-1}$)	0.69	0.69	0.69
Thermal Conductivity (W cm $^{-1}$ K $^{-1}$)	3.33 - 4.9	3.33 - 4.9	3.33 - 4.9
Bandgap Energy (eV)	2.39	3.33	3.02
Electron mobility (undoped) (cm^2 V $^{-1}$ s $^{-1}$)			
⊥ c-axis	1000	1020	450
|| c-axis	1000	1200	100
Hole mobility (undoped) (cm^2 V $^{-1}$ s $^{-1}$)	100	120	100
Electron saturated drift velocity (undoped) (cm s $^{-1}$)	~2 × 10^7	~2.2 × 10^7	~1.9 × 10^7
Hole saturated drift velocity (undoped) (cm s $^{-1}$)	~1.3 × 10^7	~1.3 × 10^7	~1.3 × 10^7
Critical (breakdown) electric field strength (~10^{15} electrons cm $^{-3}$) (MV cm $^{-1}$)			
⊥ c-axis	1.4	2.2	1.7
|| c-axis	1.4	2.8	3.0
Relative dielectric constant (100 kHz–1 MHz)			
⊥ c-axis	9.72	9.76	9.66
|| c-axis	9.72	10.32	10.03

The physical properties exhibited by SiC when treated as a set, are the most extreme for any binary compound. Some of these parameters remain constant for all SiC polytypes. However, some change, such as the bandgap energy range, and very important from an electronic point of view, which at 300K increases monotonically depending on the "hexagonality" of the polytype, this hexagonality refers to the ratio between the surrounding hexagonal structure sites and the total

number of SiC bilayers containing both a hexagonal and cubic structure in a unit cell. The hexagonality of the 3C, 6H, 4H and 2H polytypes is 0, 0.33, 0.5 and 1.0, respectively [52], [70].

4.2 Gold

When carrying out a review of the literature on biosensors for the detection of Alzheimer's, it can be observed that gold is a prevalent material among the electrodes used, but this does not only occur when trying to detect Alzheimer's. Gold electrodes are one of the materials more prevalent in the substrate of biosensors because these are already functionalized with thiolated biomolecules (-SH) through the spontaneous formation of gold–thiol bonds due to the strong affinity of sulfur with metals [71], [72]. This means that a wide range of biosensors can be made, as thiols are readily incorporated into biomolecules ranging from proteins to nucleic acids, and thiolated antibodies or antigens can be immobilized on gold, which facilitates the development of immunosensors that detect specific biomarkers [72].

5. ELECTROCHEMISTRY METHODS

Electrochemical sensors can be classified into amperometry, conductometric, impedimetric and potentiometric. In general, electroanalytical methods are considered one of the most important branches of analytical chemistry, because it allows us to determine the characteristics and the quantity of a specific analyte(s) present in an electrochemical cell [73]. The principle of operation of these sensor types is now discussed.

5.1 Cyclic Voltammetry

Cyclic Voltammetry (CV) is an electrochemical technique widely used to investigate the reduction and oxidation processes in molecular species. CV is also employed to study electron transfer-initiated chemical reactions, which includes catalysis [74].

A traditional cyclic voltammetry curve consists of traces called voltammograms or cyclic voltammograms and they can be presented under two conventions that describe the reduction-oxidation process, US convention and IUPAC convention. The convention selected for this research was US convention as shown in Figure 9. To understand CV, the x-axis represents a parameter imposed on the system, in this case the applied potential (E) the response to this parameter, the resulting current (i), corresponds to the y-axis.

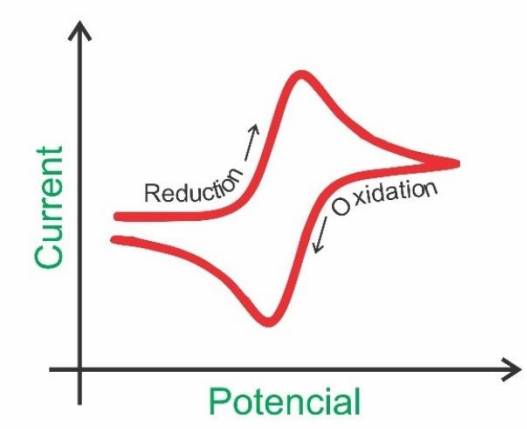


Figure 9. Cyclic Voltammetry

The experimental setup for cyclic voltammetry has a working electrode (WE) where the solution of interest is reduced or oxidized, the counter electrode (CE) which completes the circuit

with the potentiostat, the reference electrode (RE) used to measure the potential, the solution or analyte to study and the solution for the RE, which in some cases is optional [74]. This described setup is also called an electrochemical cell that, together with a potentiostat, allows a measure of electrical response generated by a chemical reaction in the solution. The data obtained can be compared with subsequent measurements and thus be able to determine the presence of a material in the sample. This will be seen more clearly when the results presented in this investigation are reviewed.

5.2 Electrochemical Impedance Spectroscopy (EIS)

Electrochemical Impedance Spectroscopy (EIS) is an electrochemical technique used to analyze the behavior of electrochemical systems and the bio-recognition events that occurs at the electrode surface, where an equivalent circuit (Randles' equivalent circuit model), based on Nyquist figures of spectroscopic impedance, is obtained [73]. EIS can be used in important biomedical diagnosis and environmental applications, such as antibody–antigen recognition, substrate–enzyme interaction, or whole cell capturing.

In practice, this process is performed by applying a potential AC waveform to a three-electrode cell and recording the resulting AC current wave. With these two AC waves, the impedance measured in ohms (as a unit of impedance) is calculated. Said impedance will have an associated magnitude and phase angle as this is a complex number. During EIS the applied AC waveform is swept as a function of frequency so that the complex frequency-dependent impedance can be measured.

EIS offers several advantages over other electrochemical techniques, because it is a steady-state technique that it utilizes small signal analysis and is able to probe signal relaxations over a very wide range of applied frequency, typically from around 1 mHz to greater than 1 MHz, using a potentiostat. EIS theory and its interpretation, while complex, is well characterized and even for new researchers in the field useful data is generally obtained [73].

As mentioned before when EIS is performed, an impedance with magnitude and phase angle is obtained. When this magnitude and phase angle as a function of frequency is represented, a Bode plot is obtained, but representation in polar and cartesian coordinates is also possible.

A particular interest in EIS focuses on its representation with a Nyquist and bode plots, which can be associated with an equivalent circuit of resistors and capacitors. Changes in the space charge region (SCR) can be followed using in EIS test, providing important information regarding surface electrode modification. In a real electrochemical system, the pattern of the Nyquist plot also includes a faradaic impedance (Z_F), which is the resistance and capacitance acting jointly at the surface of an electrode in the electrochemical cell. A faradaic impedance spectrum over a wide range of frequencies usually involves both the semicircle (frequency region in which the electrochemical process is controlled by charge transfer phenomena) and the straight line parts (at this region the electrochemical process is controlled by mass transfer phenomena) which can differ according to the respective values of the double layer capacitance (C_{dl}), the charge transfer resistance (R_{ct}) related to de passivation of the films, and Warburg impedance ($Z_{W)}$ expresses the difficulty of mass transport of the redox species to the electrode surface. The model as seen in Figure 10 also includes the solution resistance (R_u) [75].

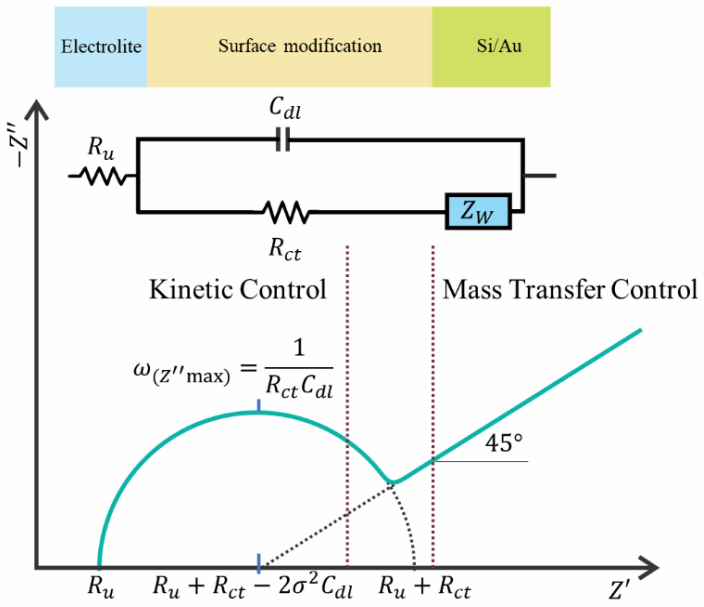


Figure 10. EIS for Randles equivalent electrical circuit over a wide frequency range.

For this research, the interest is focused on following the changes associated with surface modification, which traceability can be carried out if the change is observed in the diameter of the semicircle associated with R_{ct} as seen in Figure 10.

6. EXPERIMENTAL DESIGN AND SETUP

6.1 CV and EIS Apparatus and Electrodes

CV and EIS measurements were carried out using a VersaSTAT 4 Potentiostat shown in Figure 11. where the 4H-SiC and Au electrodes were incorporated into a conventional three-electrode cell configuration. The 4H-SiC and Au electrodes were developed in previous works by Bernardin et al. in 2018 [76] and Yaghoubi et al. in 2012 [77] respectively.

Figure 11. Photograph of the VersaSTAT 4 Potentiostat used in this research.

The fabrication and performance of the 4H-SiC electrodes was detailed in Bernardin et al. [78]; however, the experimental procedure used in this work has been refined as it follows [76]. Figure 12 shows the mask design used to produce the single-ended 4H-SiC electrodes (top) and the test structures (bottom) along with the device cross-section delineating all materials used to construct the sensor.

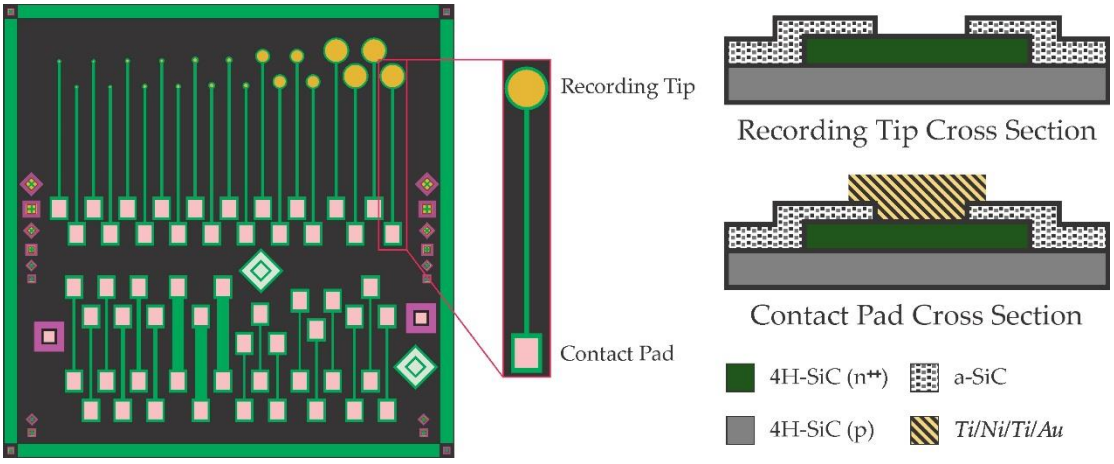


Figure 12. Single-ended electrodes (top left image) with various recording areas (diameters of 25, 50, 100, 400, and 800 µm) and test structures (bottom left) consisting of p-n diodes and resistor mesas of various length and width. Right shows device cross-section construction at both the recording tip (top right) and metal contact pad (bottom right).

4H-SiC sensor fabrication started with a p-n^+ epiwafer using various standard microelectronic fabrication methods resulting in the device cross-section shown in Fig. 10. The novel aspect of this device was the use of degenerately-doped n^+ 4H-SiC to realize metallic-like electron transport in a semiconductor mesa. Amorphous SiC (a-SiC) was used as a conformal insulator, thus resulting in a low-electrical impedance device with only SiC materials in contact with brain tissue [39].

The 4H-SiC electrodes were packaged in an electrochemical test well as shown in Figure 12 b), whereby the recording tips were exposed to the electrochemical solution while the rest of the sensor die (i.e., bonding pads) was kept dry (Fig. 13 a)) thus allowing for connection of the metal test pads to the electrochemical apparatus.

The Au electrodes (RMS roughness <2 nm) as shown in Figure 14 and Figure 15, were fabricated through the evaporation of an adhesion layer of 20 nm of Chromium at a deposition rate of 2 Å/s followed by 500 nm gold at a deposition rate of ~1 Å/s onto glass substrates [36].

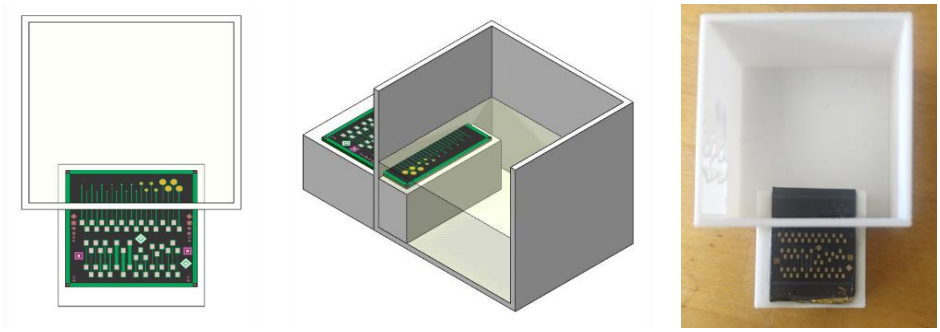


Figure 13. 4H-SiC electrode schematic showing (a) a top view of the recording tip region submerged in the electrochemical solution and (b) a 3D view of the 4H-SiC electrodes packaged in a liquid con-tainment well. (c) Photograph of the 4H-SiC electrode mounted in the test well (white).

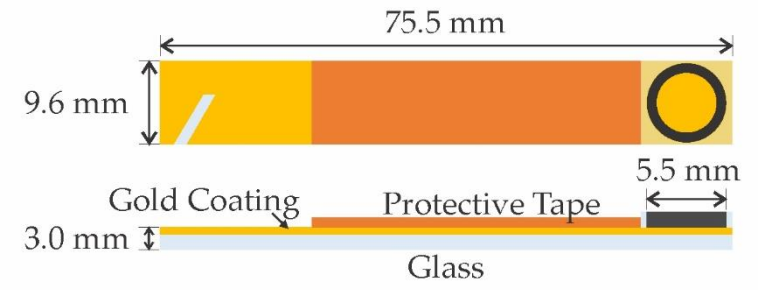
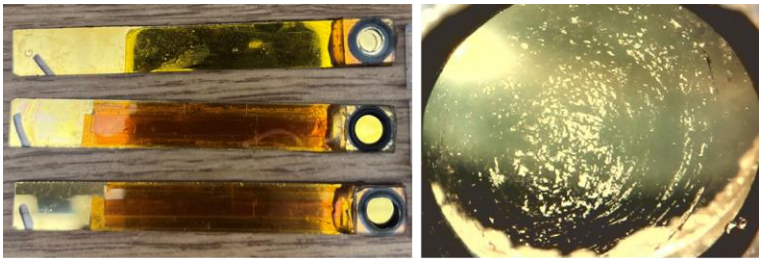


Figure 14. Diagram of gold electrodes top and side view.



a) b)

Figure 15. Photographs of a) Au electrodes (3) and b) the circular Au electrode recording tip within a liquid containment ring.

6.2 Reagents and solutions

1 mg of IgG in 0.1 ml (1 mg/ml) of Phosphate Buffer Solution (PBS) pH7.4 with 0.09% sodium azide A$\beta_{1\text{-}28}$ Rabbit polyclonal antibody and 1 mg of lyophilized solid packaged A$\beta_{1\text{-}42}$ Peptide were obtained from Abbiotec. PBS, 3-Mercaptopropionic acid (MPA), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N–hydroxysuccinimide (NHS), Concentrated Sulfuric Acid (H_2SO_4 95.0 to 98.0 w/w %), Concentrated Nitric Acid (HNO_3 70% w/w %), $K_3[Fe(CN)_6]$, $K_4[Fe(CN)_6]$, Sodium hydroxide (NaOH) and Dimethyl Sulfoxide (DMSO) were purchased from Sigma Aldrich and used in this study as described below.

6.3 Antibody and Antigen solutions

1 mg of IgG in 0.1 mL (1 mg·mL^{-1}) of PBS pH 7.4 with 0.09% sodium azide A$\beta_{1\text{-}28}$ rabbit polyclonal antibody and 1 mg of lyophilized solid packaged Aβ_{42} Peptide were obtained from Abbiotec. For the dissolution of the Aβ_{42} Peptide, Abbiotec recommends the use of 100% DMSO, and for A$\beta_{1\text{-}28}$ antibody, 0.1 M PBS solution was added until a concentration of 18.75 µg·mL^{-1} was reached.

This antibody was selected because it is a highly specific antibody [29], and it has also been used successfully on gold electrodes [28], [29]. The Aβ concentration in solution during the functionalization of the electrode surface is crucial for successful detection. If the concentration is high while the Aβ_{42} target concentration is low, the electrochemical reaction will likely not be detected. For this reason, both the antibody and antigen concentrations of 18.75 µg·mL^{-1} and 0.5

$\mu g \cdot mL^{-1}$, respectively, were taken from Dai et al. research, where $A\beta_{42}$ detection was successfully reported using a gold electrode [27].

6.4 Antibody Functionalization Protocols

This section is a description of the process developed, from the cleaning of the electrode, through the functionalization of the surface to then bind the antibody and, finally, incubating the antigen on the antibody. The Table VI summarizes the main steps described below in detail, adding some approximate data of the time required for each part of the process, with all process steps taking about 60 hours to complete.

Table VI. Functionalization steps considering time invested.

No	Step	Observation	Time (hours)
1	Cleaning	From immersion in 2M NaOH to rinse in DI Water and dry process	0.5
		CV and EIS Measurements	2-3
2	Functionalization and antibody immobilization of the electrode	Functionalization (before adding the antibody)	29
		Antibody immobilization	20
		CV and EIS Measurements	2-3
3	Antigen detection	Dissolve the antigen	0.5
		Incubate the antigen	0.5
		CV and EIS Measurements	2-3

6.4.1 Sensor Cleaning

All electrodes require periodic cleaning to remove material buildup on conductive metal surfaces. Usually, cleaning with deionized water is recommended, followed by a wash with 70% ethanol or isopropanol. According to the literature, the use of potassium hydroxide (KOH) is found to be the most effective method to leave a very clean gold surface while maintaining the electrochemical properties of the electrode [79]. However, as it can be seen in Figure 16, the structure that contains the biosensor is made of PLA (polylactic acid), a delicate material. For this reason, it was necessary to ensure that during the cleaning process the materials are not compromised. When PLA is exposed to NaOH the surface undergoes several morphology changes and, after an initial surface roughening, the surface becomes smoother again before the material dissolves. For a concentration of 2.0 mol/L (M) NaOH, the time before surface roughening occurs

is 30 minutes [80], this statement was also experimentally verified in the laboratory, observing that the structure was not compromised after the 15 minutes necessary for this experiment.

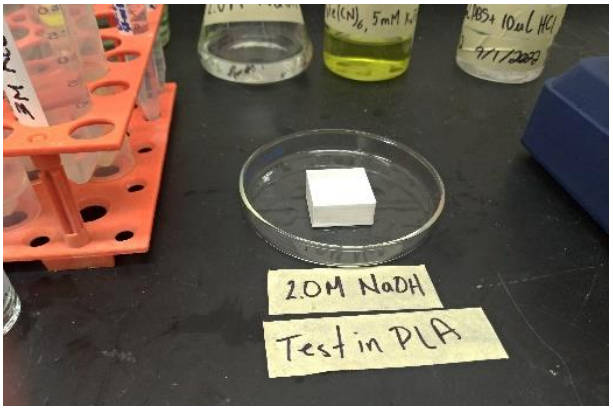


Figure 16. PLA stability test setup to ensure that exposure to NaOH in would not compromise the sensor housing.

Before starting the electrochemical experiments, the 4H-SiC and Au electrodes were cleaned as follows: The electrodes were first immersed in a 2M NaOH solution for 15 minutes and then rinsed with deionized (DI) water for 30 seconds. Next the electrodes were immersed in a 0.05M H_2S0_4 solution for 3-5 minutes and then rinsed with DI water for 30s. Lastly the electrodes were immersed in a 0.05M HNO_3 solution for 3-5 minutes and then rinsed in DI water for 30s. After cleaning was completed, the electrodes were left to air dry. Refer to Figure 20a. for reference. In Table VII the solutions used in the cleaning step are listed.

Table VII. Chemical agents used during the cleaning process.

M	Formula	Name	Molarity	Molar Weigh [g/mol]:	Volume Prepared [mL]:	Quantity needed [g]:	Volume Needed [µL]:
2.0	$NaOH$	Sodium Hydroxide	-	39.997	100	8.0	-
0.05	H_2SO_4	Sulfuric acid	18.4	98.08	100	-	272
0.05	HNO_3	Nitric acid	15,7	63.01	100	-	318.47
0.1	PBS	Phosphate buffered saline	-	411.04	100	5 tablets of 0.01M PBS	-
0.005	$K_4Fe(CN)_6$	Potassium hexacyanoferrate (II) trihydrate	-	368.34	100	0.18417	-
0.005	$K_3Fe(CN)_6$	Potassium ferricyanide	-	329.44	100	0.1646	-

Posterior to air drying, the electrodes surfaces showed a cleaning improvement, as observed in Figure 17.

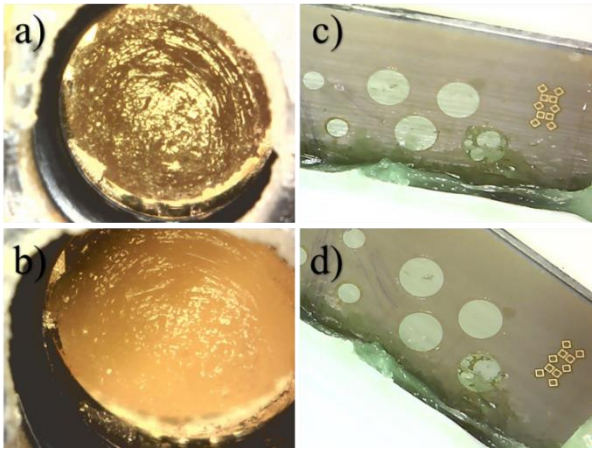


Figure 17. Au and 4H-SiC electrodes a) and c) Before Cleaning, b) and d) after Cleaning.

The cleanliness of the electrodes can not only be verified visually as confirmed by Figure 17, but also an improvement in the response of the electrodes can be confirmed by means of CV and EIS techniques, as observed in Figure 18.

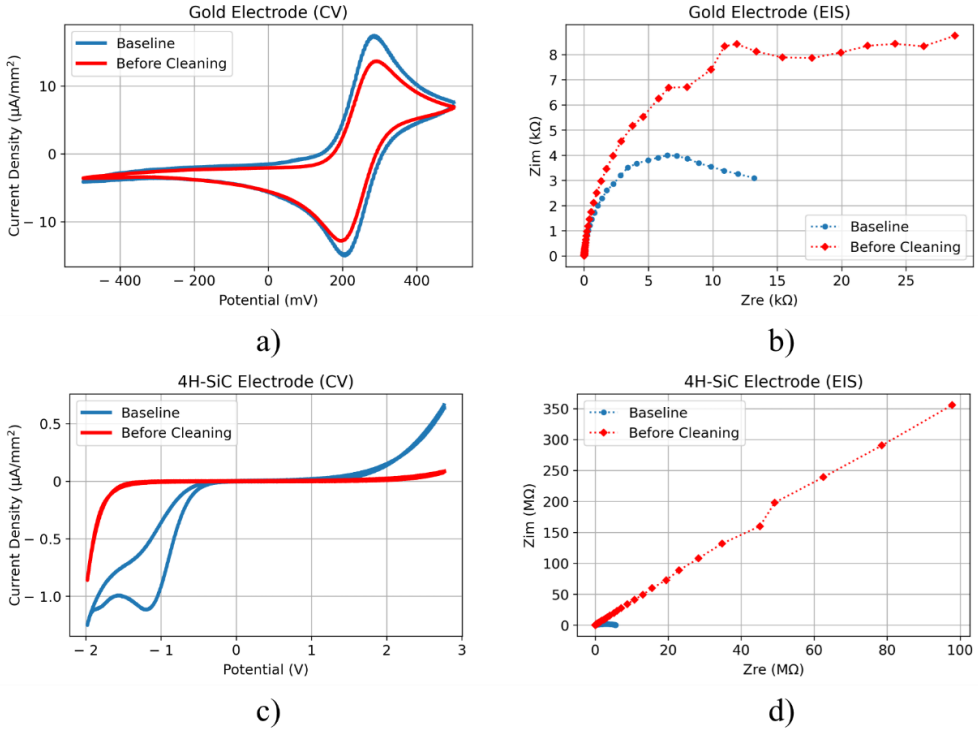


Figure 18. Before and after cleaning response in Au Electrode a) CV b) EIS and SiC electrode c) CV d) EIS

6.4.2 Electrochemical Cells

In order to properly and accurately test each electrode a 3-probe configuration was used for the electrochemical cell setup with an Ag/AgCl reference electrode (RE), Pt wire as the counter electrode (CE), and our electrode under test (Au or 4H-SiC) as the working electrode (WE) as shown in Figure 19. All electrodes were then submerged in the liquid electrolyte. The electrolyte solution possessed a pH of 7.4 with the following chemistry: 0.18417g of $K_4Fe(CN)_6$, 0.1646 g of $K_3Fe(CN)_6$, 100mL 0.1 M PBS.

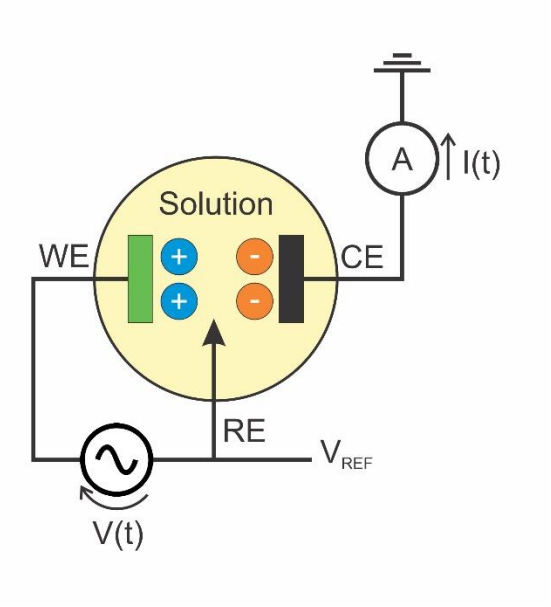


Figure 19. 3-probe configuration electrochemical cell

6.4.3 Procedure & Measurements

Once the electrodes were appropriately cleaned, they were characterized via CV and EIS measurements, as shown in Figure 20. These readings were defined as the baseline for each electrode's response. CV settings for measurements were defined with respect to the material used: in the Au electrode case the sweep voltage was from -0.5 V to 0.5 V, while in case of 4H-SiC this material has a much wider water window, so the voltage was varied from -1.98 to +2.77 volts. For EIS the initial frequency was 10 kHz with an end frequency of 0.1 Hz. for both electrode types. Upon establishing the baseline performance of each electrode, the electrodes were subsequently functionalized using the following procedure:

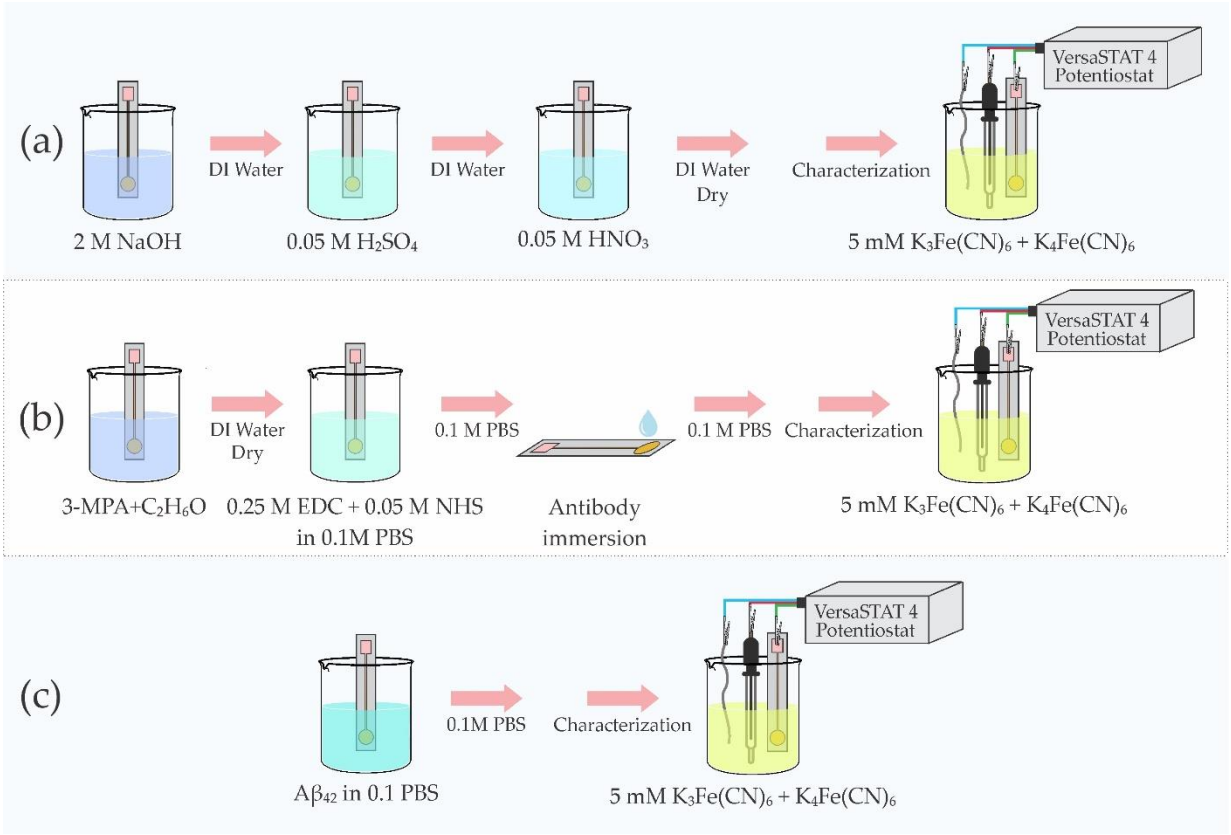


Figure 20. Procedure a) Cleaning, b) Functionalization and antibody immobilization, c) Antigen Detection

To functionalize the electrodes, they were first immersed in a 1mM solution of 3-MPA in Ethanol for 24 hours in the dark to achieve a self-assembled dried 3-MPA-SAM (self-assembled monolayer) layer, then rinsed with DI water and dried gently. Afterwards, the exposed end of the 3-MPA-SAM was functionalized by treating the electrodes in a solution of 0.1M PBS containing 0.25M EDC and 0.05M NHS for 5 hours, rinsed with DI water and dried gently. Next, the electrodes were immersed in 20 mL of Aβ antibody solution ($A\beta_{1\text{-}28}$) with a concentration of 18.75 µg/mL for 20 hours at 4°C, rinsed with 0.1M PBS and stored at 4°C. Once functionalized CV and EIS measurements of the sensors were performed, to validate their response to antibody exposure. This procedure and its pathways for activation reactions are shown in Figure 21 where a) a carboxylated thiol-SAM is formed on the electrode surface, b), EDC addition forms an activated O-acylisourea surface. A number of different reactions can occur as dimerization to form c), formation of N-acylurea d) (undesired reaction) or with NHS forming e), the succinimide intermediate (desired reaction). The anhydride c) can also form the succinimide intermediate e)

with NHS, or it can be hydrolyzed back to the carboxyl groups a). Finally, after addition of Antibody in covered surface f) is formed.

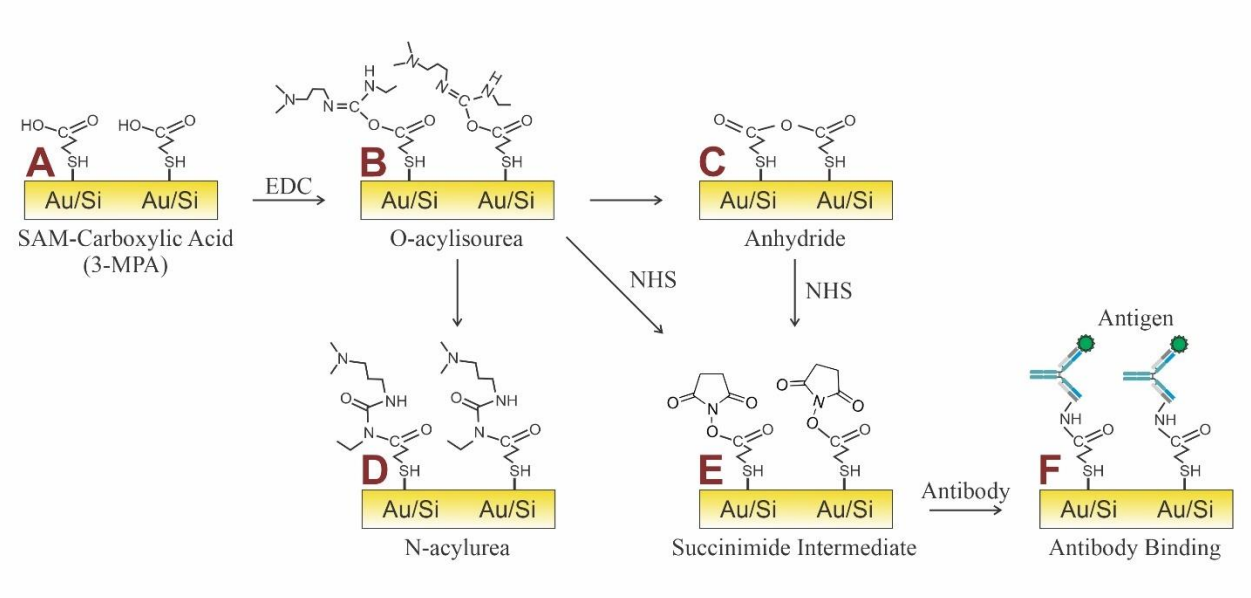


Figure 21. Pathways for activation reaction

After antibody response measurements, a solution of Aβ_{42} Antigen dissolved with DMSO, to prevent aggregation, was diluted with 0.1 M PBS solution to reach 0.5 μg/mL of Aβ_{42} concentration. The diluted solution was incubated for 30 minutes at the top of each biosensor, as recommended in the literature [23]. Prior to CV and EIS measurements of the sensor response to the Aβ_{42} Antigen, the biosensor was rinsed in 0.1M PBS. CV and EIS measurements were then performed in an electrolyte solution described earlier (10 mL solution of $K_4Fe(CN)_6$ and $K_3Fe(CN)_6$ of 5mM in 0.1 M PBS). In Table VIII the solutions used in the functionalization, antibody immobilization and antigen detection steps are listed.

Table VIII. Chemical agents used during the functionalization, antibody immobilization and antigen detection process.

M	Formula	Name	Molarity	Molar Weigh [g/mol]:	Volume Prepared [mL]:	Quantity needed [g]:	Volume Needed [µL]:
0.001	$3-MPA$	3-Mercaptopropionic acid	13.36	106.14	200	-	14.97
0.25	EDC	N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride	-	191.7	21	1.0	-
0.05	NHS	N-Hydroxysuccinimide	-	115.09	21	0.1208	-

0.1	PBS	Phosphate buffered saline	-	411.04	100	5 tablets of 0.01M PBS	-
0.005	$K_4Fe(CN)_6$	Potassium hexacyanoferrate (II) trihydrate	-	368.34	100	0.18417	-
0.005	$K_3Fe(CN)_6$	Potassium ferricyanide	-	329.44	100	0.1646	-

7. RESULTS

For clarity it is best to divide the following electrochemical data graphs into a comparison of "steps" that lead to a conclusive analysis of the electrochemical sensor under study (please see Figure 20 for reference). In the last section device cleaning (baseline), antibody functionalization and $A\beta_{42}$ functionalization steps were described. After each step the sensors were tested in the aforementioned electrochemical solution and setup. With the intention of having a better comparison, Figure 22 shows the data obtained for the Au electrode and Figure 23 displays the data for 4H-SiC electrode 3.

As seen in Figure 20a, the CV baseline in the gold electrode shows a reversible reaction with the reduction and oxidation peaks at 290 mV and 210 mV, respectively. After the immobilization of the antibody, the peaks were shifted to 310 mV and 180 mV, and the magnitude of the current was increased. The CV results also show a higher current level after the introduction to Aβ ($A\beta_{1-28}$) antibody. Still, the shape of the curves shows a fully reversible redox reaction on the gold electrode. The EIS results in Figure 5b reveal more details about the differences between the tested samples. The radius of the semicircles at the higher frequencies increased from the baseline to the antibody sample, indicating larger charge transfer resistance in the electrode with the immobilized antibody. While a slight decrease in the radius was observed after the incubation of the antibody, the tail of the curve at lower frequencies shows a distinct feature. As seen in the Nyquist plot, the tail of the impedance at low frequencies represents the mass transfer limitations. It is clear that in the presence of $A\beta_{1-28}$, the mechanism of redox reaction in potassium ferricyanide is more affected by the interaction of the antibody to the immobilized antigen.

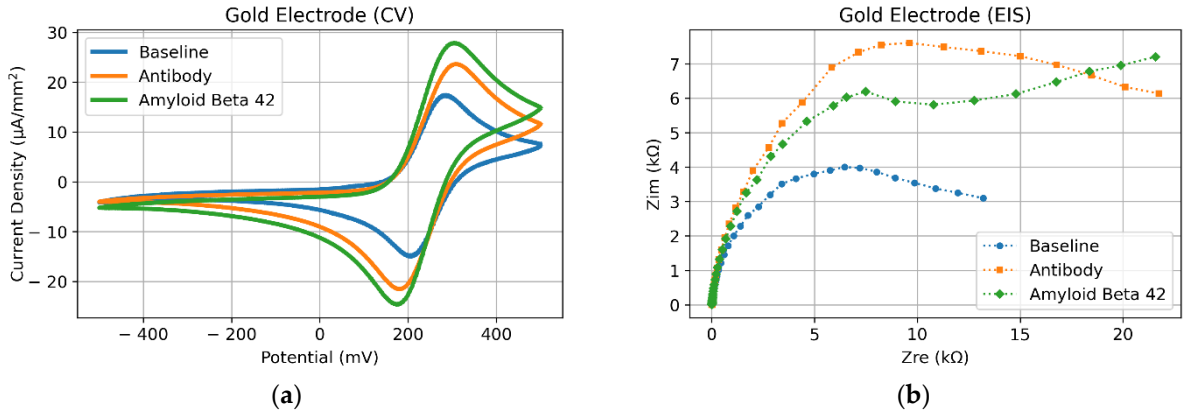
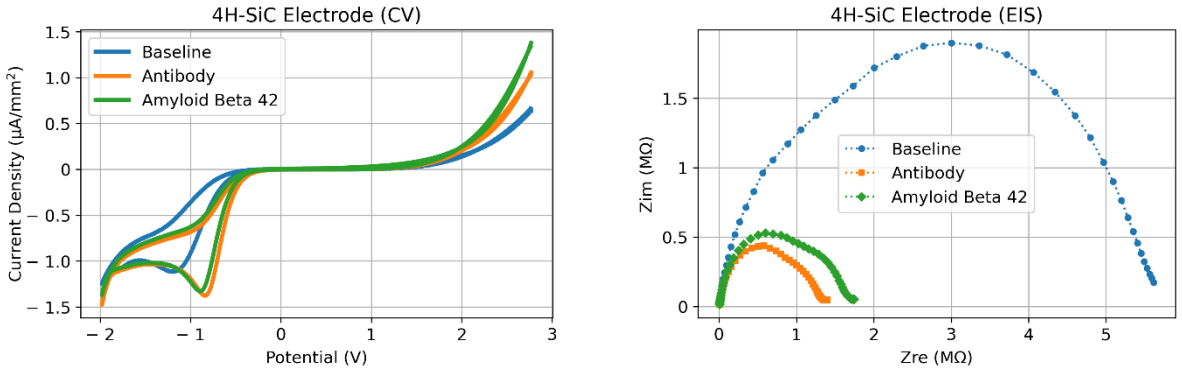


Figure 22. Gold electrode response at each step of the electrochemical sensor functionalization. a) CV response and b) EIS response.

The experimental results from the 4H-SiC electrode are mainly focused on electrode 3 which is considered to be a successful result and shows a totally different response (Figure 23). First, despite the wider voltage range in the CV experiment, there are only oxidation peaks at negative voltages and no reduction peak. However, the exponential increase in the current at the positive voltages represents the Butler–Volmer model (limitation by the kinetics of the reaction) [81]. Such a rectifying response is expected from a wideband gap semiconductor as the electron donation rate is significantly lower than the electron acceptance rate by the electrode. In addition to the domination of the oxidation process in the SiC electrode, immobilization of the antibody had a significant impact on the oxidation peak, shifting it from −1.19 V in the baseline to −0.85 V in the electrode with the immobilized antibody. However, the peak voltage did not change after the incubation of $A\beta_{1-28}$. Unlike the gold electrode, the EIS results from the 4H-SiC electrode (Figure 23b) show the domination of the semicircle response and almost no tails at the lower frequencies. This again shows that due to the semiconducting property of the electrode, the kinetic rate of the reaction dominates the response of the sensor. While the baseline shows a relatively large semicircle, after adding the antibody, the radius decreased significantly, implying a faster kinetic rate. However, the antigen had a reverse response resulting in a slightly larger semicircle radius (slower reaction rate).



(**a**) (**b**)

Figure 23. 4H-SiC electrode 3 response after each electrochemical sensor functionalization step. (a) CV response and (b) EIS response.

Below, Figure 24 shows the response of 4H-SiC electrodes group, in a general analysis it can be said that Electrode 1 presents contamination on the surface that does not allow a correct baseline curve. In case of Electrode 2 the correct addition of $A\beta_{42}$ was not achieved. On the other hand, for electrode 3 and 4 all the stages were successful.

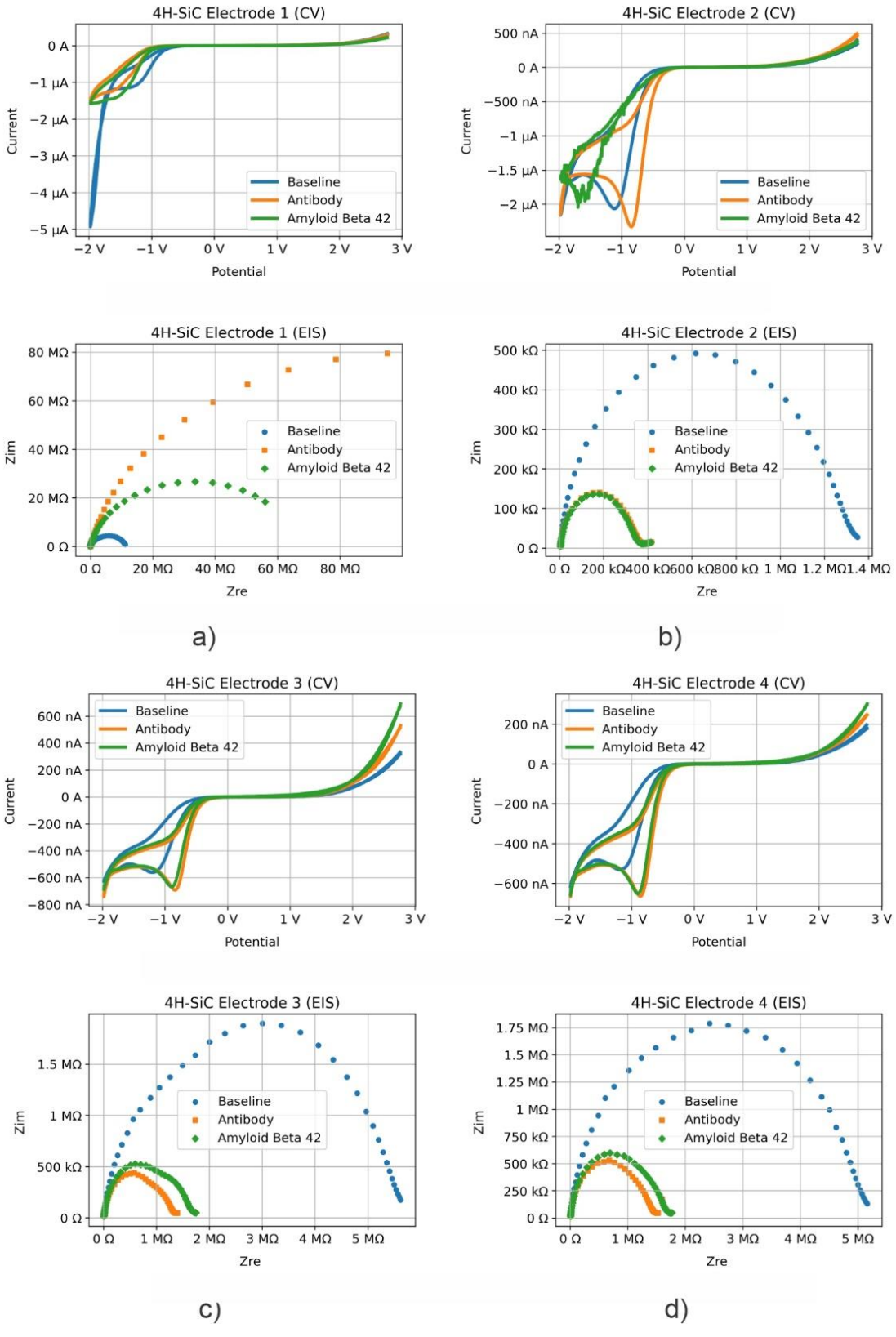


Figure 24. 4H-SiC electrode response at each step of the electrochemical sensor functionalization. CV and EIS results for a) Electrode 1, b) Electrode 2, c) Electrode 3 and d) Electrode 4

Comparing the two electrodes, the most important observation was that $A\beta_{42}$ was detected by both the Au and SiC sensors, albeit at different voltages and with different amplitudes. It appears that the gold electrode was sensitive enough to utilize a lower voltage input and provide a clearly measurable response compared with the SiC sensor. It should be noted that the contact surface of the 4H-SiC electrode had a diameter of 800 µm compared to 5.5 mm in the case of gold. While the CV results are reported based on the current density, a much smaller electrode surface area in the SiC electrode resulted in a much larger impedance (MΩ range) than that in Au (kΩ range). Nevertheless, incubation of the biomaterials had the opposite effect on the impedance of the gold and SiC by increasing the impedance in the gold electrode and decreasing it in the SiC.

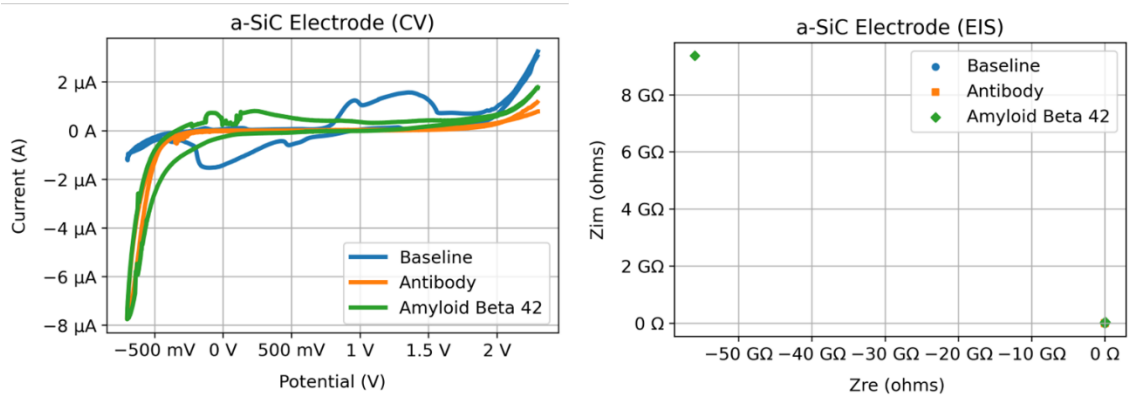


Figure 25. CV (Left) and EIS (Right) results for a-SiC Electrode 1.

It is worth mentioning that tests on other types of electrodes such as Amorphous SiC (a-SiC) were carried out; however, a stable baseline could not be established, functionalization was not possible and therefore detection was not possible. An example of these results is shown in Figure 25 above for completeness.

7.1 Products

As a product of this research the paper *SiC Electrochemical Sensor Validation for Alzheimer Aβ42 Antigen Detection* [82] was published in the open access journal on the science and technology of small structures, devices and systems, *Micromachines*.

8. CONCLUSION AND DISCUSSION

In conclusion, this thesis research presented preliminary sensor response data from two electrodes made using Au and 4H-SiC, respectively. Their surfaces were functionalized and tested for their suitability as effective electrochemical biosensors for Alzheimer Antigen ($A\beta_{42}$) detection. The results presented here demonstrated that both sensors resulted in the detection of $A\beta_{42}$, a highly important finding. The Au surface (larger) displayed a larger signal response compared to 4H-SiC (smaller area), but the results presented here beg for more exploration on the topic of SiC specificity in the context of the desired application. However, it should be pointed out that the data is very preliminary and SiC neural probe electrodes were used for these tests, which were not of the same form fit as the Au electrodes (800 μm tip diameter vs. 5.5 mm for Au)

Most importantly, this research contributes to the science of defining diagnostic measurements of Alzheimer's $A\beta_{42}$ detection. Future work in this area by our research team will involve the use of other metal oxides to baseline the electrochemical biosensor response so that the best candidate material can be further developed to detect AD.

The ultimate research objective, if we consider the big picture, is to determine if this type of fast CV sensor can be used as a saliva-based sensor which would truly revolutionize AD biosensor research, but in order to do that first it is necessary to determine if it is possible to successfully functionalize the electrode surface for its use in this type of application and tune the testing methods to validate the functionalization. In this work we have accomplished these requirements necessary to move to the next stage of development, and with that we have developed originals protocols not reported in the literature that could potentially benefit the development of not only this, but other similar concept-based technologies.

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