AUSense Results Document
Presented by: The American University in Cairo

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2. Summary

Adalimumab is the largest selling drug worldwide that relieves the symptoms of rheumatoid arthritis. Rheumatoid arthritis is a long-term, chronic disorder that results in inflammation of the synovium, the tissue that lines the inside of joints. Unfortunately, treatment is quite expensive and a large portion of the patients do not benefit or lose treatment benefit as they take the drug (23-46% lose benefit over time). As the global prevalence of rheumatoid arthritis approaches 1%, it is vital that we measure the concentration of the adalimumab drug in the patient's blood to monitor and tailor how much of the drug should be given to a patient.

We are a team from the American University in Cairo, composed of students from diverse backgrounds ranging from chemists to mechanical engineers. This semester we have worked long hours together to develop a hand-held electrochemical biosensor that measures the concentration of Adalimumab both accurately and quickly in less than 5 minutes. We as a team have truly been motivated not only by the challenge of this task but also by its medical importance; this device can be of great help for patients suffering with rheumatoid arthritis.

3. Biosensor System and Assay

3.1 Molecular recognition and assay reagents

Similar to other well-established immunoassays such as ELISA, our team's biosensor relies on the interaction between an antigen and the antibody. Unlike ELISA, electrochemical detection methods were utilized for our biosensor. The antigen used being TNF-α, while Humira is the antibody employed in the biosensor. All the dilutions made were with phosphate-buffered saline (PBS, pH 7.4). Furthermore, carbon screen printed electrodes functionalized with gold nanoparticles alongside 11-mercaptoundecanoic acid ($C_{11}H_{22}O_2S$) aided in the immobilization of TNF-α on the surface of the electrode. The immobilization of TNF-α on the electrode was ensured by SEM/EDS analysis. The SEM/EDS results[1] confirmed the immobilization of TNF-α through the appearance of nitrogen on the electrode immobilized with TNF-α and the absence of nitrogen on the electrode before immobilization.

Hexammineruthenium(III)chloride ($Ru(NH3)_6^{3+}$)used serves as a redox cycling agent. Upon the introduction of the blood sample spiked with Humira, which is the target antibody, the immobilized TNF-α complexes with Humira forming an insulator layer that impedes electron transfer between the redox species and the electrode surface reducing the electrochemical current generated in the absence of the target antibody. Moreover, exploiting this observed phenomenon, a calibration curve could be generated in order to establish rapid and accurate ways of determining the concentration of Humira present in the blood samples of patients at hospitals or any testing facilities.

[1] See appendix 10.1

3.2 Physical transduction

Electron transfer resulting from the interaction of the redox species with the electrode produces a current in the microampere range. The electrode is inserted in a cartridge/electrode holder, which is then connected to a small handheld potentiostat that can measure current in the pico ampere range (this is to improve accuracy). The potentiostat is connected to a computer, which utilizes a certain software program that runs the tests.

3.3 Cartridge technology

The disposable device wherein the sample fluid is treated is a basic carbon screen printed electrode, however, coated with several layers each contributing to a specific function. The basic carbon electrode is first treated with gold nanoparticles, as this gold coating has various benefits, such as enhanced electrical conductivity, good binding to thiol groups and good chemical stability. After being treated with gold, the electrode is then also treated with 11-mercaptoundecanoic acid ($C_{11}H_{22}O_2S$). This acid facilitates in the immobilization o f TNF-α on the surface of the electrode. This sequence of layering results in the final disposable electrode wherein the blood sample resides. The figure below illustrates this sequence:

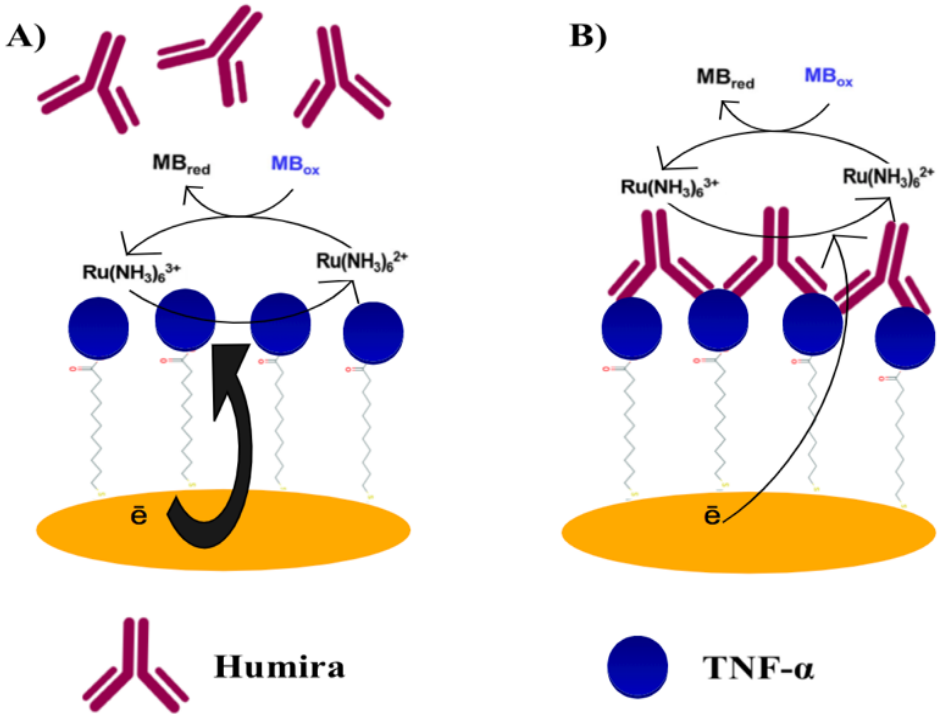

Figure 1. Diagram of the electrochemical design of the working electrode. **(A)** In the absence of Humira, a significant electrochemical signal is generated (**B**) In the presence of Humira, an insulator layer is formed due to the TNF-Humira complex that forms on the surface and hinders electron transfer thereby reducing the electrochemical signal.

3.4 Reader instrument and user interaction

The circuit board required was designed to test a difference in the potential difference, so a potentiostat was the optimum circuit to be used for the task. After searching and comparing between a “do it yourself” potentiostat or already available one in the market, we have decided on the EmStat Pico Development Kit. The kit is offered with a PSTrace software for electrochemistry which helps with running the experiment by creating optimal settings and a script to control the EmStat. The used language for the device is MethodSCRIPT scripting language. This language allows the developer to program a readable script for users.

The device is designed to be user friendly in that the user places the chip inside the device easily and the results appear on the computer after. There is one input and one output on opposite sides of the device with no tricky mechanisms. The chip is placed in the input side and the output side has a USB connection with the computer. It is manufactured using laser cutting and made from acrylic with no sharp edges that could hurt the user.

Specifications:

Table 1. Main Specifications of EmStat Pico Development Board

Main specifications of EmStat Pico Development Board	
Dimensions	90 x 65 mm
Power	(Micro) USB or 2x AAA battery
Main connections	– BT900 Bluetooth module Bluetooth v4.0 dual mode (BT and BLE) – Micro-USB
Cell connections	screw terminals (2.54 mm pitch) and LEMO (EPG.0B.305.HLN)
Arduino compatibility	Footprint for Arduino MKR series
Storage	8 GB Micro SD card
Real Time Clock	On-board IC: S-35390A-T8T1G With CR1225 3V coin cell battery compartment (battery not included)
Buttons	4 buttons for: – EmStat Pico Reset – EmStat Pico Download – EmStat Pico Wake – Bluetooth Module reset

4. Novelty and Creativity

4.1 Already available

The concept of electrochemical redox cycling is already available in the literature. The redox cycling agent, Hexammineruthenium(iii)chloride, is reduced by a bias potential and then oxidized back to its original form. This process is continuous as the Hexammineruthenium(III)chloride is simultaneously regenerated. Moreover, this redox cycling results in an electrochemical system. If a target antigen is present in the sample, it binds to the antibody immobilized on the surface of the working electrode. Thus, the antigen-antibody complex formed acts as an insulating layer that impedes the electron transfer to the electrode. By limiting the rate of electron transfer, the current signal produced decreases. Moreover, the reduction in the current produced is inversely proportional to the concentration of the antibody present in the plasma sample. For instance, if there is a high concentration of the antibody then the reduction in current will be more significant due higher impedance. Therefore, it is possible to correlate the reduction in current with the concentration of the Adalimumab. The device consists of a screen-printed electrode (SPE) and a potentiostat. SPE's have been around for decades and consists of a reference, working and counter electrodes. The electrodes are often created of carbon, which is chemically inert and produces a low background current. Nevertheless, other materials such as gold are now used as they can easily be modified with self-assembled monolayers. This is because gold has a strong affinity to the thiol group. Moreover, the potentiostat used was purchased from PalmSens. The Emstat Pico Development Kit was purchased and the electrical components and circuits required some setting up.

4.2 New developments

The device we created is the first to utilize the concept of electrochemical redox cycling and impedance to measure the concentration of Adalimumab. The team chose all the chemicals that would be on the carbon screen-printed electrode (SPE). The SPE was specifically customized by Orion to consist of a working electrode covered in gold nanoparticles and functionalized with 11-mercaptoundecanoic acid ($C_{11}H_{22}O_2S$). The counter electrode was made of carbon while the reference electrode was chosen as silver/silver chloride. Later, based upon preliminary experimentation we chose to immobilize 10ng TNF-α on the working electrode. Also, based upon preliminary experimentation, 1mM of Hexaammineruthenium(III)chloride was chosen as the most suitable concentration of the redox reagent. Moreover, a very small plasma volume of only 10microlitres is required. The device we have created is the only one in literature that works in under 5 minutes and produces highly accurate results of adalimumab levels in blood plasma

5. Analytical Performance

- Sample Volume: 10 microliters
- Dilution: 15 microliters of redox species is added

Experiments are carried out by mixing 10 microliters of the sample with 15 microliters of the redox solution to give a total volume of 25 microliters, all of them pipetted onto a screen printed electrode.
Samples containing Adalimumab showed a decrease in current measured using the potentiostat, hence confirming the hypothesis that once the Adalimumab binds to the TNF there is a reduction in the electrochemical signal.

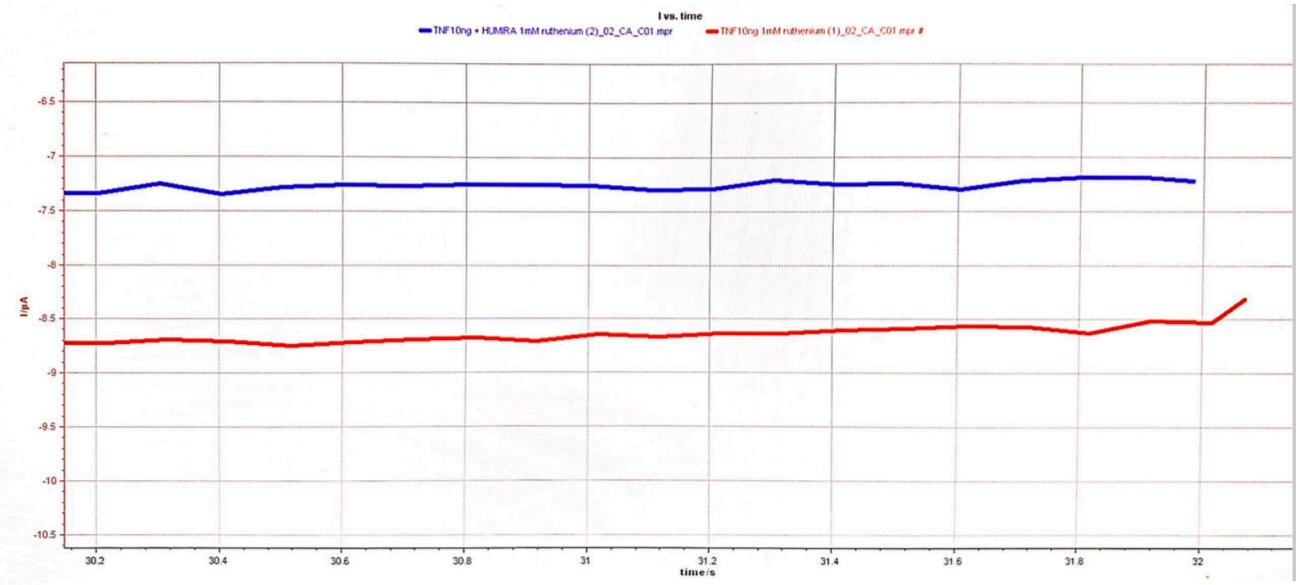

Figure 2. Chronoamperometric results. The red line represents the electrode immobilized with 10ng TNF and 1mM Hexammineruthenium(III)chloride. The blue bottom line represents the electrode with 10ng TNF, 2μg/ml 1mM Hexammineruthenium(III)chloride.

The time needed to take the reading can vary depending on the desired accuracy of the measurement, however, it is not less than one minute and does not exceed four minutes.

6. Translation Potential

This Business Plan (hereinafter "BP") covering major pillars/considerations for proposing the developing of a Biosensor that detects Adalimumab (commercially known as "Humira") in blood plasma in less than five minutes (used mainly to treat Rheumatoid Arthritis "RA"). We have split the BP in three distinct sections that cover most aspects of the project (Stakeholder Desirability, Business Feasibility and Financial Viability).

6.1 Business model canvas

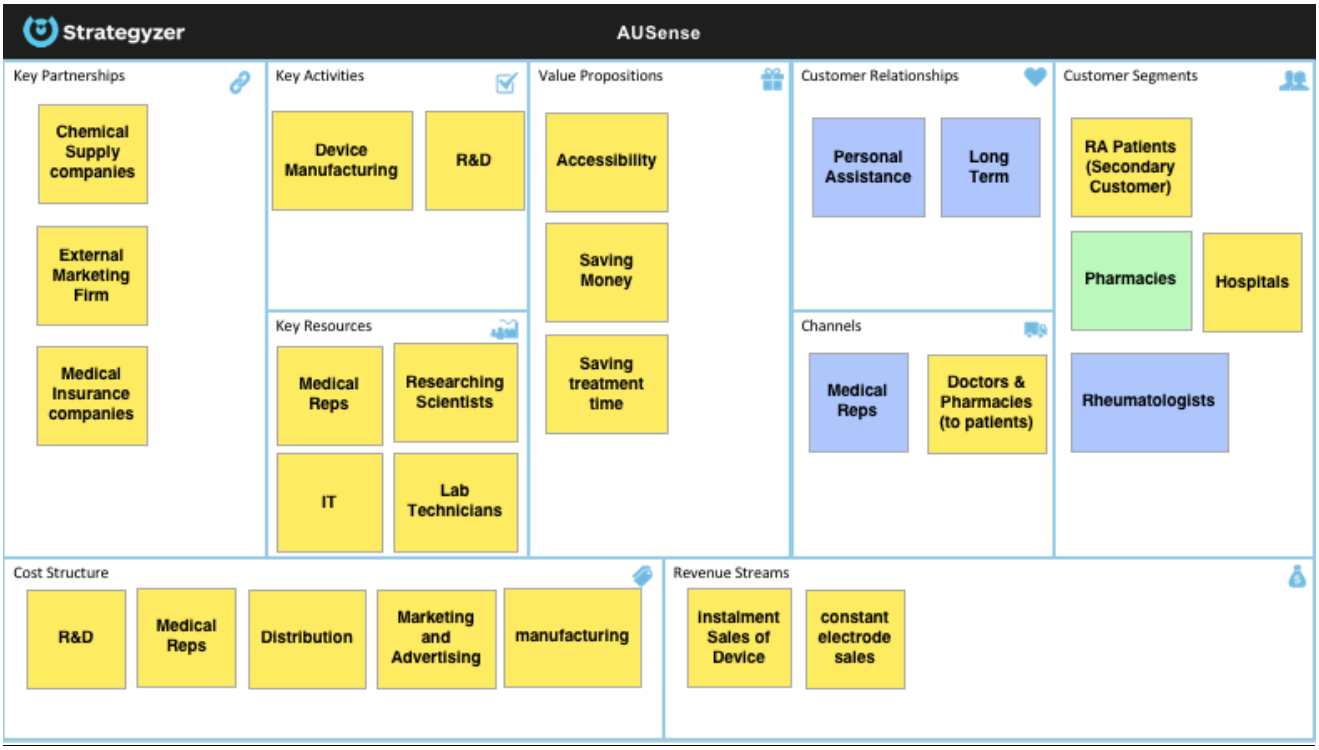

Figure 3. Business model canvas

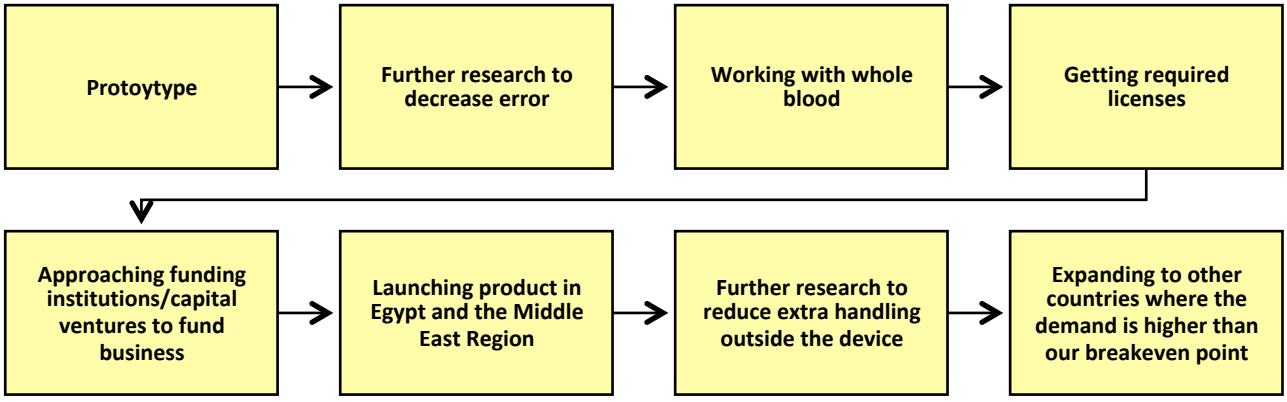

Figure 4. Product development plan

Table 2. Stakeholder's desirability analysis

Stakeholders Desirability	
Category	**Comments**
Originality of the solution and need in the market	• RA patients' number hover around 1% of the world's population (about 70 million); while Adalimumab is considered one of the main treatments, due to lack of information regarding number of patients, we are basing our calculations on a primary basis that 20% of patients are treated with adalimumab. • The dose of Adalimumab is to be decided by the Rheumatologist basis set of expensive lab analysis (about €200 and € 500 in Europe and Egypt, respectively). Test results takes long time to come out (7-45 days). • State of the art testing of Adalimumab is available but requires 1:19 dilution ratio, 15 minutes of time and is measures a smaller range than the device being proposed. This device "The Quantum Blue® Adalimumab" is not widely available in the market, so we consider our main competition to be the medical tests available and being used. • Due to the above, most patients depend on physicians' experience on the greatest part to prescribe the Adalimumab dose. • Proposed solution provides a permanent/portable device and disposable cartridges that produce results within few minutes at much cheaper cost. • Future adaptability to mobile Apps that keep test results in one box and enables on-line communication to physicians.
Potential Stakeholders	• RA Patients (receive optimal treatment at reduced cost and less pain). • Insurance companies (pay less and less frequent to same patient). • Rheumatologists (prescribe proper dose first time). • Pharmacies (new business that used to go to laboratories). • Investors and Fund Managers (value creation for prospective investors).
Monetary Value	• Cost of the assembled unit in Egypt is about € 1,000 (one-time investment). • Cartridge cost is for €4 (€2 for electrode and €2 for layering). • Used cartridges shall be returned to re-layer for a cost of €2. • Plan to reach 10% of the RA patients (1.4 million) to run six tests a year and generate ***net margin*** of €1/cartridge (about €8.4 million per year). ➢ *Economies of scale at time of mass production shall create an opportunity to reduce units and cartridges' cost.*
Support	• Prices of medical tests are averages of multiple medical labs asked • Currently, the frequency of testing in Egypt happens biannually at most according to 65 RA patients asked.
Threats	Fierce negative publicity from current labs (as they will lose expensive business). However, we can easily bring to project's side by installing units in their facilities.

Table 3. Business feasibility study

Business Feasibility	
Category	**Comments**
Resources	• Financing from Investors and Mutual Funds (basis sound financial viability). • Researchers. • Software Developers / IT. • Lab Technicians. • Lab Space + Machinery required.
Activities	• Activities to be done by the team/employees: ➢ *Device and cartridge manufacturing.* ➢ *Provide suitable facility to cater for re-usable cartridge and related layering activities.* ➢ *Medical Representatives to visit Rheumatologists and pharmacies to promote for device benefits to physician and patient).* ➢ *Initial training to customers on how to use the device and the concept of re-layering.* • Activities to outsource: ➢ *Distribution activities (per-cartridge arrangements with Pharmaceutical Distributors).* ➢ *Initial marketing (secure reach to different customers/consumers).* ➢ *Venture capital specialists (prepare prospectus for offering).* ➢ *Legalizing the project (Obtain license and related patents, if needed).* ➢ *Call center provider to gauge patients' vibes and physician feedback.*
Partners	• Medical Insurance Companies (as previously stated). • Investors and Fund Managers (as previously stated). • Chemical companies (to secure constant/reliable/timely supply). • AUC (provide lab space and machinery for a pre-determined deferred fee to be paid from the first trench of free cash flows).
Threats	Rapid technological advancement can make this project obsolete. However, continuous R&D works is needed to cope with such advancements.

Table 4. Financial viability analysis

Financial Viability	
Category	**Comments**
Costs Projection	• Direct cost of the Biosensor is €1,000, €2 for the cartridge and €2 for re-layering. ➢ *Biosensors revenue and cost will be recognized with every layering till fully paid.* ➢ *Cost of the Electrode/Cartridge will be booked as Fixed Assets and depreciated.* ➢ *Cost of the re-layering will be booked as Cost of Goods Sold upon sale.* • Mass production shall surely enjoy quantity discounts and supply chain efficiencies. • Provision shall be built for % of devices that will either get damaged; or never redeemed.
Sales Price	• No margin will be recognized for the sale of Biosensors (will be sold at cost). • Margins will be recognized with every Re-layering transaction. • Given the wide difference between current lab analysis cost (€ 200-€500) and the absence of several cost elements (such as marketing, research and others), sales price shall be calculated via reverse formula to yield €1 per re-layer and generate net profit before tax of about € 8.4 million per year.
Market Size	• We plan to reach 10% of market size per year (around 1.4 million patients). However, extensive marketing/awareness programs shall take place prior to launching. • We forecast market size to grow at rate of 10% per annum (as physicians see more value to use this new tool and as the business expands to other countries).
Revenue Streams and Business Strategy	• Biosensors will be distributed to pharmacies, hospitals, medical labs and clinics on consignment (i.e., not paid for upfront). • With every cartridge re-layering, pro-rata revenues & cost of Biosensor will be recognized till fully paid for (example, €100 unit will be paid by the customer through €1 per cartridge for the first 100 cartridges re-layering).
Support	• Costs mentioned are based on the cost of the prototype (subject to significant decrease)
Threats	Over sale of layered cartridges to customers increases the risk of layer expiration and hence, risk of returns (however, customer size study should be carefully performed).

7. Team and Support

7.1 Contributions of the team members

- Namir Elkhouly and Mohab Amr are both mechanical engineering students who were responsible in designing, manufacturing, and assembling the device
- Habiba Tarek and Karim Rafik are electronics engineering students who were mainly responsible for assembling the circuit and making all connections
- Omar El Sayyad, Laila Elfeky, Ghada Adly, Dania Abdel Dayem, and Omar Khoshala are all chemistry major students who established the electrochemical method of detecting Adalimumab in blood plasma.

7.2 People who have given support

- Dr. Hassan Azzazy is the chair of the chemistry department at AUC and is our supervisor. He has been assisting us throughout and helping us resolve any issues related to the development of our biosensor.
- Dr. Ehab El Sawy is a chemistry professor in AUC who guided us in developing the idea and method of detection and provided us with the basic electrochemical principle behind this biosensor.

7.3 Sponsors

Our team did not use outside sponsors to help with funding.

8. Final Remarks

We are truly pleased and proud with the device we have created nevertheless we encountered some time related issues, as the chemicals and equipment that we needed would often take months to arrive due to strict regulations in Egypt. Although we wanted to experiment with different redox reagents and other chemicals, unfortunately we were unable to do so as chemicals took an extremely long time to arrive. In the future, we aim to further explore the efficiency of different redox reagents and further optimize the parameters of the chemicals we used. Moreover, we would also want to functionalize the carbon screen printed electrode ourselves.

We would like to thank Dr. Hassan Azzazy for assisting us throughout this process and bringing us the opportunity to participate in this international competition, as it was a true learning experience for all of us. We would like to also thank all the professors who gave us their time to answer any of our specific questions in their area of expertise. Finally, we must thank Dr. Ehab ElSawy in specific as his help has truly been invaluable; Dr. Ehab ElSawy gave us his time and taught us the advanced electrochemistry required for creating this device.

9. References

Dutta, G., & Lillehoj, P. B. (2018). Wash-free, label-free immunoassay for rapid electrochemical detection of PfHRP2 in whole blood samples. Scientific Reports, 8(1). doi:10.1038/s41598-018-35471-8

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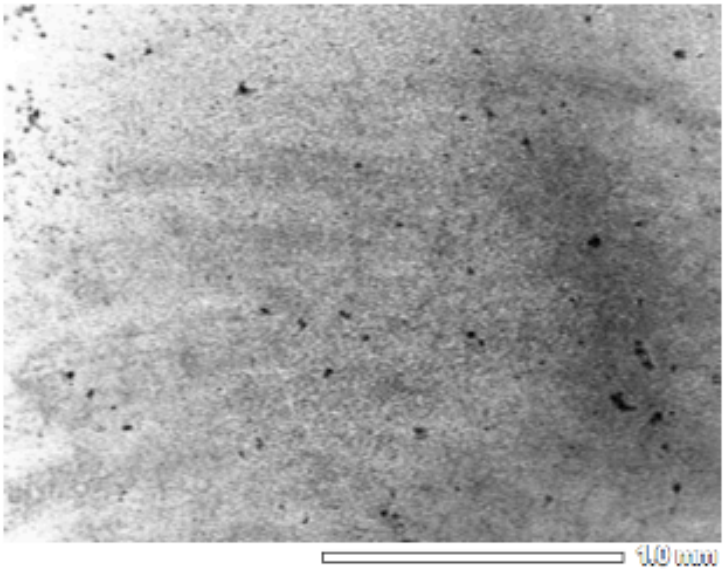
10. Appendix

10.1 SEM/EDS Results

1) Before TNF-α immobilization; electrode with gold nanoparticles functionalized with 11-mercaptoundecanoic acid ($C_{11}H_{22}O_2S$).

View001

JEOL 1/1



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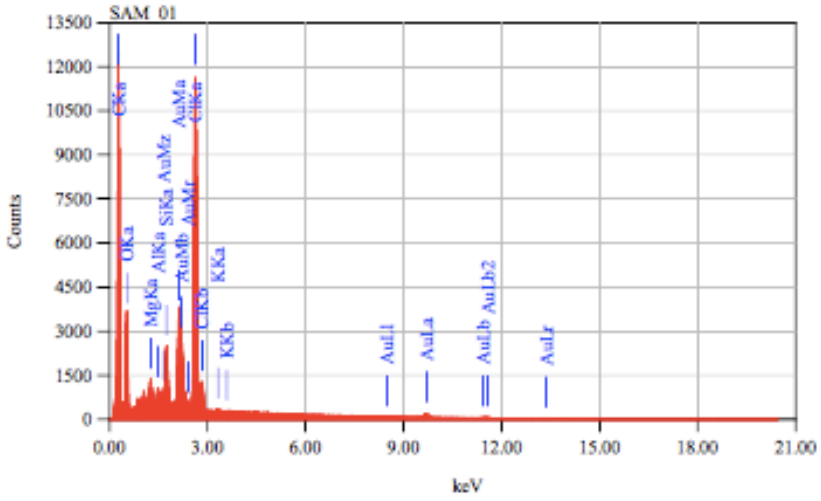
Mag.	: x 50
Date	: 2019/07/31
Pixel	: 512 x 384



Acquisition Parameter

Instrument	: JCM-6000PLUS
Acc. Voltage	: 15.0 kV
Probe Current	: 7.47500 nA
PHA mode	: T3
Real Time	: 54.76 sec
Live Time	: 50.00 sec
Dead Time	: 8 %
Counting Rate	: 11027 cps
Energy Range	: 0 - 20 keV

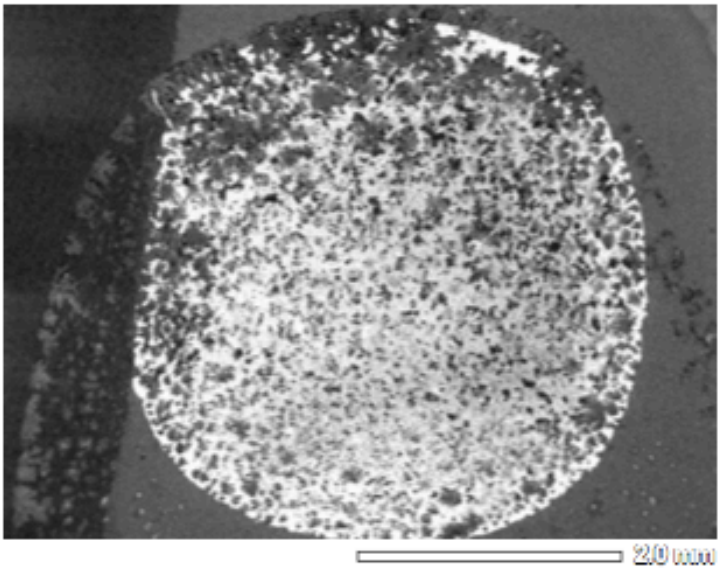
Thin Film Standardless Standardless Quantitative Analysis
Fitting Coefficient : 0.0626

Element		(keV)	Mass%	Counts	Sigma	Atom%	Compound	Mass%	Cation	K
C	K	0.277	39.06	70215.86	0.10	69.71				2.3445
O	K	0.525	4.70	21851.29	0.04	6.30				0.9063
Mg	K	1.253	1.05	6533.61	0.03	0.93				0.6796
Al	K	1.486	0.64	3795.31	0.02	0.51				0.7161
Si	K	1.739	2.93	16792.69	0.04	2.23				0.7342
Cl	K (Ref.)	2.621	29.48	124244.08	0.13	17.82				1.0000
K	K	3.312	0.19	703.15	0.02	0.11				1.1593
Au	M	2.120	21.94	29514.33	0.20	2.39				3.1333
Total			100.00			100.00				

2) Electrode with immobilized TNF-α

View005

JEOL 1/1



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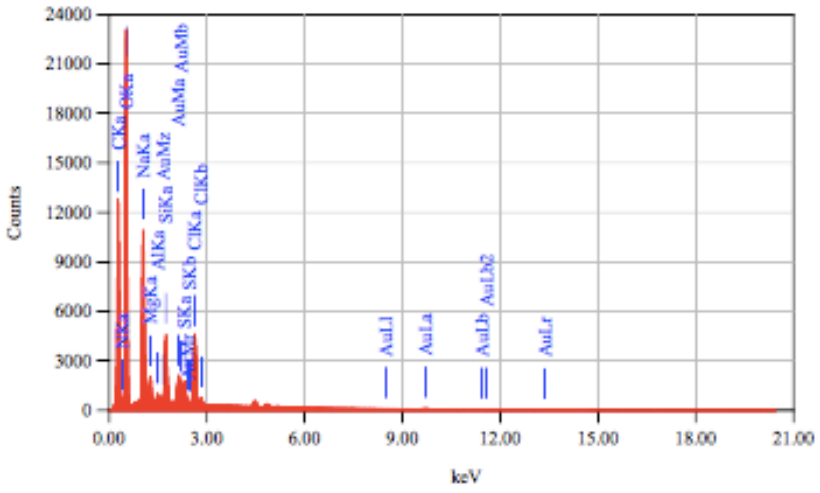

Acquisition Parameter
Instrument : JCM-6000PLUS
Acc. Voltage : 15.0 kV
Probe Current: 7.47500 nA
PHA mode : T3
Real Time : 55.84 sec
Live Time : 50.00 sec
Dead Time : 10 %
Counting Rate: 13566 cps
Energy Range : 0 - 20 keV

Thin Film Standardless Standardless Quantitative Analysis
Fitting Coefficient : 0.0272

Element	(keV)	Mass%	Counts	Sigma	Atom%	Compound	Mass%	Cation	K
C K	0.277	34.57	78495.88	0.08	51.17				2.5867
N K*	0.392	0.72	2943.32	0.01	0.91				1.4347
O K (Ref.)	0.525	24.60	144481.28	0.08	27.34				1.0000
Na K*	1.041	11.89	90744.16	0.06	9.19				0.7693
Mg K	1.253	1.67	13071.72	0.03	1.22				0.7498
Al K	1.486	0.48	3573.50	0.02	0.32				0.7901
Si K	1.739	5.13	37226.19	0.05	3.25				0.8101
S K	2.307	2.40	13874.02	0.03	1.33				1.0161
Cl K	2.621	8.75	46572.14	0.06	4.39				1.1033
Au M	2.120	9.78	16616.10	0.13	0.88				3.4571
Total		100.00			100.00				