Team Results Document
SensUs 2019

Navigating through the tempests of arthritis with the good hope of a better life - solution for a fast detection of Adalimumab

Team members

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1 Summary for the SensUs website

The LxUs biosensor has the purpose of allowing an easy, cheap and fast measurement of the plasma concentration of Adalimumab (ADL), an active component in drugs used to treat rheumatoid arthritis (RA).

The biosensor is built upon three major components: the detection system, the gold nanoparticles (Au-NP) solution, and the smartphone application.

The working principle is based upon the spectrophotometry technique which allows transduction of changes in optical properties of the 60 nm dimater Au-NP, functionalized with anti-ADL antibodies, as they bind to ADL.

The Au-NP solution is contained in a plastic cuvette allowing an easy and fast mix of nanoparticles and plasma. The detection system is comprised of a 405 nm laser, as the light source, and a photodiode as a detector, allowing for the transduction of the light transmitted through the cuvette into an electric signal. This signal is then amplified, processed and fed into an Arduino board with a Bluetooth module streaming of the results to a smartphone application that displays and records them for future monitoring.

2 Biosensor System and Assay

2.1 Molecular recognition and assay reagents

The LxUs biosensor working principle is the measurement of changes in the optical properties of a solution of gold nanoparticles (Au-NP) functionalized with biotin as they bind to Adalimumab (ADL) molecules. These changes are proportional to the concentration of ADL. The absorbance peak of Au-NP shifts to higher wavelengths as the particles bind to a ligand (the ADL) [1–3], increasing their radius. By increasing or decreasing the concentration of ADL, the absorbance value changes proportionally.

For this purpose, Au-NP, 60 nanometre diameter, pegylated (PEG 5000) and biotin terminated, were employed (Sigma-Aldrich, product code 808911) together with anti-ADL antibodies for molecular recognition. For assays, lyophilized human plasma (Sigma-Aldrich, product code P9523) and anti-TNF-/*alpha* (Adalimumab) (Biovision Incorporated, catalog number A1048) were used.

2.2 Physical transduction

The biosensor is based on the spectrophotometry technique to obtain the concentration of ADL present in the sample. For that, a collimated laser diode (Thorlabs, part number CPS405) with a 405 nm wavelength is used with a corresponding photodiode. The beam crosses a cuvette (Sigma-Aldrich, product code C5291) containing the sample and the transmitted intensity is then converted to an electric potential by the photodiode. This signal is amplified by a negative-feedback amplifier circuit, sent to an Arduino UNO board and the corresponding sample concentration transmitted via Bluetooth to a smartphone application. Schematic of the device apparatus is show in figure 1.

The measured values allow calculation of the sample's concentration of ADL bound to the Au-NP. Obtaining data in order to create a calibration curve enables the computation of the sample's ADL concentration on the Arduino UNO board.

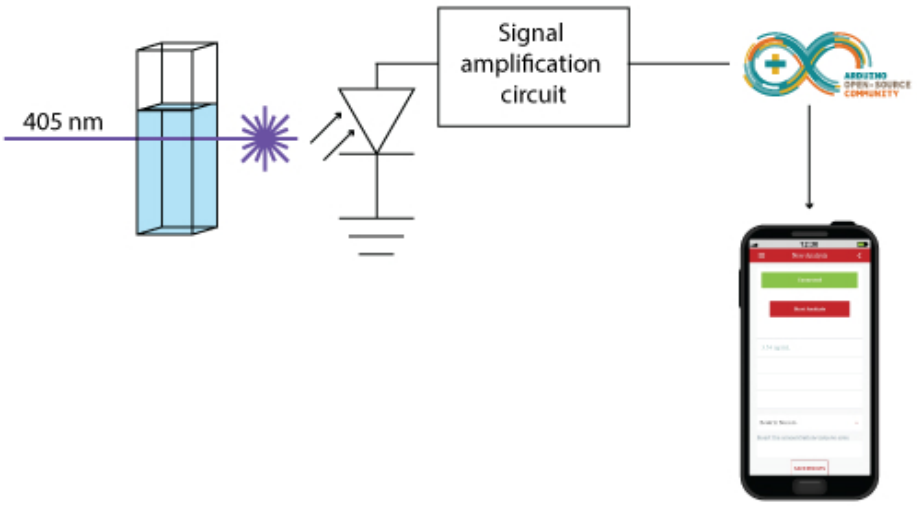


Figure 1: Schematic of the LxUs biosensor apparatus. The laser intensity transmitted through the sample solution is converted into an electric signal, amplified and processed. Afterwards, the user receives the concentration measured on a smartphone application..

2.3 Cartridge technology

Regarding the handling of the fluid, other than the test tubes and the micropipette's tip, we opted for the usage of polystyrene cuvettes (Sigma-Aldrich, product code C5291) with two polished windows for the light measurement step, placed in a cuvette holder.

2.4 Reader instrument and user interaction

To ease the interaction between user and device, an intuitive smartphone application was created. It allows creation of patient profiles and storage of their respective test results. As such, any Android

smartphone can be used as a reader instrument for the LxUs biosensor. The application was developed using OutSystems. Communication with the biosensor is performed via Bluetooth, namely a module connected to the Arduino UNO board.

The user needs only to insert a small cuvette containing the solution of plasma, Au-NPs and ADL in the equipment. By pressing a button in the application, the ADL concentration is shown on the screen. When the application is accessed, the main screen presents three options: New Patient, Patient List, New Analysis.

The "New Patient" screen allows you to create a new profile. Once you have finished filling your new patient's profile out, just click save to add it to your Patient Profile List.

The "Patient List" screen lists all the patient profiles you have created so far. Clicking on one of the names opens the Patient Profile of your choice, providing you with even more options. If you click the "More Details" option, you will be shown the most important information of your patient's data.

The "New Analysis" option takes you to a screen which allows communication with the LxUs biosensor. In there, you are able to turn on the Bluetooth of your mobile device and begin the sample analysis by simply pressing a button. Once the sample testing finishes, the results will be shown on the screen.

3 Novelty and Creativity

3.1 Already available

Since nanoparticles (NPs) concentration is usually determined in terms of mass [4], we took into consideration the more established mass concentration methods: Atomic Absorption Spectroscopy (AAS), Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), Ultraviolet-Visible (UV-Vis) Spectroscopy, Near Infrared (NIR) measurements, Quartz Crystal Microbalance (QCM).

AAS measures the concentration of ground-state atoms by quantifying the absorption correspondent to the allowed transitions from ground to excited states. The radiant power absorbed is related to the absorption coefficient, which in turn is related to the mass concentration by the Beer-Lambert law [5]. This method has been recently used to confirm the formation of a nanocomposite consisting of silverpolyethiophene (PT), by dispersing it in a colloidal solution with Ag-NP [6].

Through the ICP-MS method, plasma is used to ionize or excite elements, that are then focused and travel through a quadrupole mass analyzer at a rate determined by the mass/charge ratio until they reach the detector. It can thus be used to measure both mass and number concentration [7]. This technique was recently coupled with Flow Field-Flow Fractionalization to quantify and determine the size of CeO2 (cesium dioxide) NPs in suspension [8].

UV-Vis Spectroscopy (190 nm to 750 nm) is a method used for quantitative analysis and compound identification. It is based on Absorption Spectroscopy, where the absorption results from electronic transitions to higher energy states and/or vibrational and rotational transitions, determined by the type of bonds. In this case, the energy absorbed determines de absorbance that is proportional to the concentration of the analyte. The total absorbance of a solution is the sum of the different absorbances for each compound [9]. This method has been applied for chemical analysis of dilute samples and liquid-dispersed nanomaterials, where sample preparation and infrared (IR) absorption took only a few minutes in total [10].

In the NIR region, electronic, vibrational and rotational transitions can still be generated, however, in electronic transitions, the NIR and visible regions become hard to distinguish. On the other hand, it is relatively easy to distinguish between NIR and IR regions in vibrational transitions. Absorption in the NIR region has been maximized by coating gold nanospheres with hyaluronic and oleic acids (HAOA) using a novel method of bioproduction without increasing their toxicity [11].

QCM is a quantitative mass measuring device using quartz plate resonators as sensitive as microbalances. Quartz is a piezoelectric crystal and it has a frequency resonance dependent on thickness. The working principle of QCM is given by the Sauerbrey Equation that relates mass density deposition with frequency variation. This implies an existing linear relation between the measured frequency and added mass [12]. QCM has been used to measure rheology of colloidal suspensions showing that the sensor response correlates to changes in the suspension yield stress [13].

3.2 New developments

We opted for the UV-Vis absorption spectrophotometry and took advantage of the Beer-Lambert Law to obtain the desired concentration of the Au-NPs with anti-ADL - bound to ADL, by relating the transmittance with the concentration of a solute in a given sample. The biosensor we built aimed at the combination of optical, chemical and electronic principles we gathered in a device which is able to not only measure the desired concentration of ADL in the given sample but also transmit it as reliable information to the user of our intuitive app through an Arduino board.

4 Analytical Performance

For sample preparation, in an Eppendorf (or equivalent equipment) add 180 μL of water and 20 μL of the plasma sample. Separately, prepare a solution of gold nanoparticles in water at a concentration of 0.5 $\mu g/mL$. In a cuvette, add 1 mL of the gold nanoparticles solution. Following that, add the 200 μL of the plasma in water solution. Mix the preparation (using inversion or the up and down technique with a micropipette, for example). Make sure there are no particles or bubbles in the solution. Place the cuvette in the equipment. The cuvettes' polished surfaces must face the laser beam component. A total of twenty samples were prepared for the assays performed, each took approximately three minutes to complete.

Assays were done with solutions of known concentration (from 0.5 to 10 $\mu g/mL$) and the electric potential was measured through the Arduino UNO board. For each concentration, three measurements were performed. Concentration was then plotted as the dependent variable and the measurements' electrical potential average as the independent variable. With a linear regression, the relationship between both quantities was obtained (figure 2).

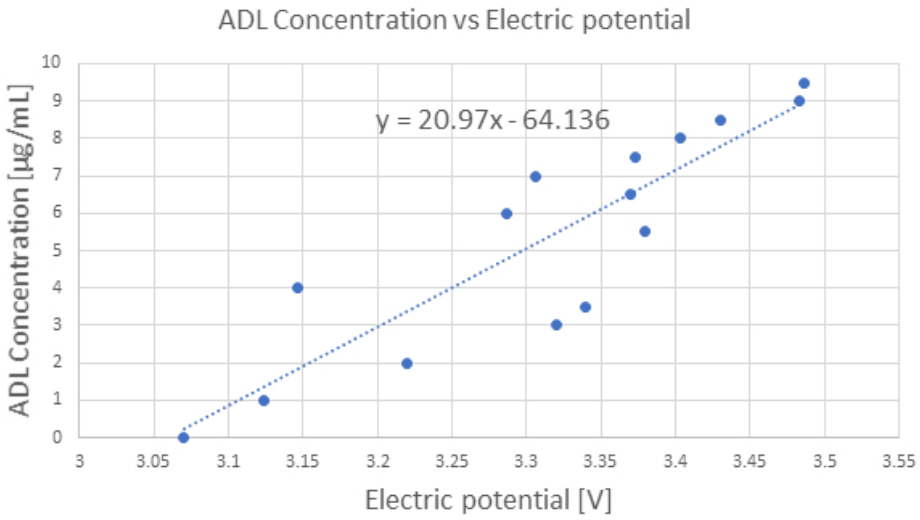
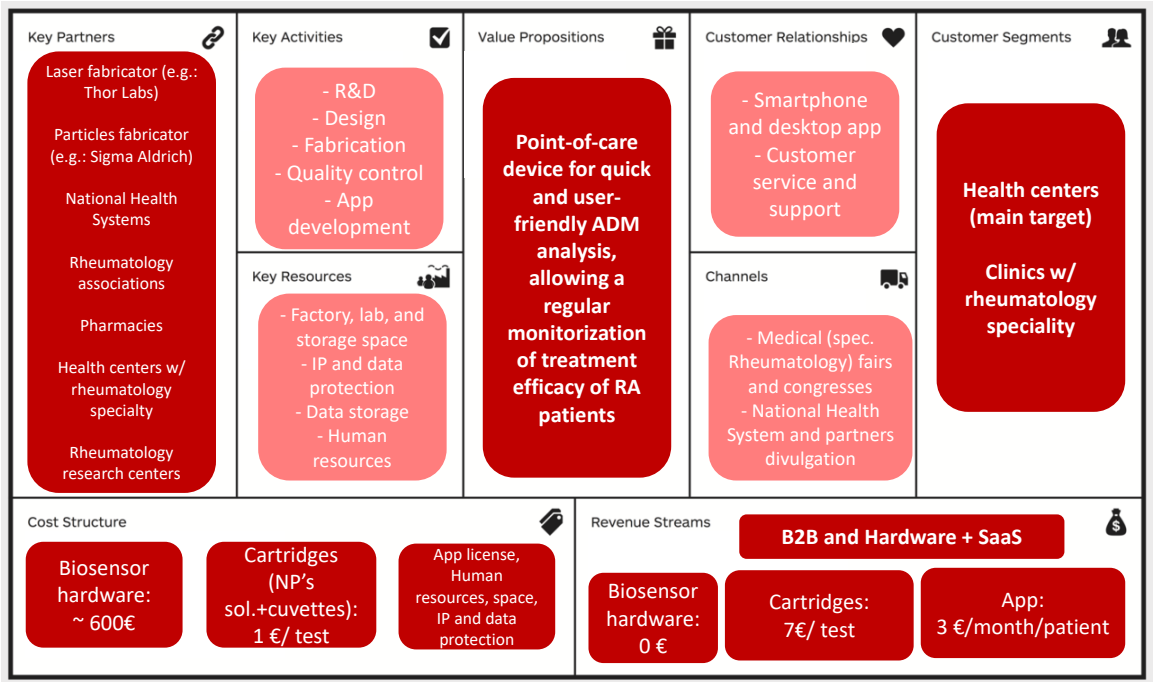


Figure 2: The LxUs biosensor calibration curve. The biosensor shows an approximate linear response in the 0-10 $\mu g/mL$ Adalimumab concentration range, encompassing the therapeutic target steady state concentration of 5-8 $\mu g/mL$.

5 Translation Potential

5.1 Business model canvas



5.2 Stakeholder Desirability

For RA patients (our users), the main problem nowadays is the lack of a regular monitoring of their treatment, specifically of the efficacy of ADL. In fact, patients taking this drug as a treatment for RA, typically visit their doctor only two or three times per year. Consequently, often patients are taking ADL without knowing if the drug is working or not. Furthermore, in the specific case of Portugal, it adds that there are very few health centers with rheumatology specialty (about 7 in the whole country), and that they are only in big cities. Hence, patients living away from these cities, have a hard access to proper follow-up. There is, therefore, the need of a way of providing these patients an easy access to treatment check-ups.

The centers with rheumatology specialty (involved stakeholders, possibly our partners) have the problem of, due to their low number, having a very high number of patients, which leads to a long waiting time and to a non-optimal service.

The health centers (general non-specialized, exist in Portugal in a high number, and across all of the country) (our customers), don't have a problem *per se*. However, since they are included in the Portuguese National Health System, they serve as a support for the specialized centers, therefore they can be used in order to have a better management of patients flow.

Our value proposition is, in fact, a point-of-care device for quick and user-friendly ADL analysis, allowing a regular monitoring of treatment efficacy of RA patients. By having this device in health centers, the care of RA patients, namely in Portugal, can overcome its limitations and have various benefits: providing an easy access to patients living away from the big cities, less waiting times in the specialty centers, and the usage of health centers for these patients that only need a simple check-up (being not necessary to go to a specialized doctor). In sum, our solution would allow RA patients to have their ADL levels regularly checked-up, in a center closer from their homes; and the health system would gain a better management of these patients (being that only patients who really need to go to a rheumatology doctor – in case ADL levels are not according to the desired ones – would go).

5.3 Financial viability

Our proposed product involves a material cost estimation of 1€ for the cartridges (1 test kit: cuvette + NP's - Gold Nanoparticles - solution) and 603€ for the biosensor (per apparatus). We also have the assembly costs, which we estimate being 0,15€/min for the cartridges and 4€/h for the biosensor, based on the average wage of a pharmacist and on the minimum wage (for the simple assembly of the components), respectively. Our business model is the following: we will offer the biosensor hardware for the purchase of a minimum amount of 150 test kits, and then sell extra test kits. Additionally, we will also charge a monthly subscription fee for our app, where all the results and analytics will be displayed, both for the clinician and for the patient.

The size of our first target market (Portugal) is very promising, being that 10 thousand million euros were spent in 2018 in the National public health service. Regarding the corresponding sales prices, our biosensor can be sold separately for 750€, but since our business model includes only the purchase of the minimum amount of test kits, these will be sold for 7€ per unit. Given that, each health care centre will have to pay a minimum of 1050€.

Lastly, the financial outlook is very positive: assuming we can reach 5% of the health care centres in the 1st year of sales, which corresponds to 18, we will have a total cost of 17679,6€ and 1220,4€ of revenues. By the 2nd year, assuming 20% of the health care centres, we would reach 15879€ of revenues. By the end of the 3rd year, we aim to reach 50% of the total number of health care centres, corresponding to 182 facilities, and 56665,2€ in revenues.

5.4 Business Feasibility

In order to develop, manufacture and scale up our sensor, we need the following key resources: a factory, lab and storage space; IP protection (a patent covering our unique innovative technology and/or method); data protection services; data storage services (servers and cloud); and human resources (pharmacists, biomedical engineers, physics engineers, hardware technicians, IT technicians/engineers, among others).

One of our key activities will be Research & Development (including: particles test and optimization, laser – sensor electronics, signal amplification electronics, sensor calibration, processing algorithms, Arduino's control system, and Arduino – app interface). We will, however, have other key activities: design (app interface, and device packaging), fabrication (including: electronics assembly, laser – sensor alignment and setup, and Arduino – app Bluetooth interface), and quality control.

In order to scale up and have a viable business, we need to count with some strategic partners: a laser manufacturer (e.g.: Thor Labs), a particles manufacturer (e.g.: Sigma Aldrich), National Health Systems, rheumatology associations, pharmacies, and health centers w/ rheumatology specialty. We can also perhaps have a partnership with rheumatology research centers, since they can gain from the data we eventually can provide (having into account GDPR protocols), and we can leverage from their expertise in research in order to further develop our technology.

6 Team and Support

To develop our biosensor, we gathered a team of 15 individuals with expertise in different areas. Then we divided them in four work groups: Management and Entrepreneurship, Hardware, Biotech and Application.

6.1 Contributions of the team members

Management and Entrepreneurship
Team with the function of dealing with the oversight of the work developed, but also to communicate with the different faculty offices necessaru. Additionally, due to SensUs entrepreneurial perspective it was also this team that communicated with outside entities that could help us. Composed by Nuno Gonçalves (Team Captain), Rita Maçorano (Team Captain) and Francisca Canais. Between them, the team was responsible for oversight of technical and entrepreneurial components of the project, communication with teachers, faculty members and external entities, travel logistics, social media and marketing.

Hardware
Team responsible for biosensor development, which included the detection scheme, the control electronics and its physical platform. Composed by Afonso Santos, Ana Nascimento, Bruno Santos, Rafael Almada and Rita Alves. Together they developed the equipment, worked on the Arduino sketch, performed calibration tests and analysed the respective data. Bruno Santos was the main responsible for the team.

Biotech
Team responsible for the nanoparticle solution that would allow the best optical analysis and best binding rate to ADL. Most of this team work was equally shared by its members. Composed by Alexandra Sousa, Maria Quitério, Mariana Figueira and Pedro Francisco. Alexandra Sousa was the main responsible here.

Application
The objective of this team was to develop an user friendly mobile phone application that could work as the display for the analysis results and additional information. Composed by Beatriz Donato, Catarina Pinto and Francisco Sequeira. They developed a mobile application using OutSystems that is capable of communicating with the LxUs biosensor through Bluetooth, create patients' profiles and store data. Beatriz Donato was the main responsible for this part of the project.

6.2 People who have given support

Besides professor Hugo Ferreira, our team responsible, and professor Catarina Reis there were many others that helped us. Professor Manuel Abreu and his team at Laser and Optics Laboratory in FCUL, were really important for the biosensor development, they never gave up on us and when things looked difficult, we always found a way out of it. Additionally, professor Guiomar Evans advised us in the electronics of the biosensor, for which we are very thankful.

We also want to thank to Duarte Teixeira, who helped us understand how the Portuguese health care system works, and how would be a path of a AR patient in this system.

A lot of different Portuguese institutions helped us spread awareness regarding RA problems. One of the main contributors was the Portuguese Society of Rheumatology through their president Prof. Helena Canhão that helped us understand how can we improve the life of people with this disease.

6.3 Sponsors

One of our big sponsors was TecLabs, the innovation center at FCUL, that kindly arranged a space for us to use as headquarters. Additionally, all of the staff gave us support with the bureaucratic work and even helped us creating our logo.

7 Final Remarks

2019 was the first time a portuguese team from the Univerity of Lisbon participated in the SensUs competition. It was a great learning experience in many different topics.

At first sight we weren't sure of what to expect from the competition or what challenges would be ahead of us. Of all the challenges we surpassed, one stood out, the communication with the suppliers and dealing with internal bureaucracy were major obstacles to pass, it delayed our work in many weeks. Without the materials to test and build we couldn't work. As stated on feedback moments, our team was on hold for a critical amount of time. This lock didn't allow us to explore all the options and tests that we had planed for. Consequently this delay had effects on how our research was done, therefore notorious influence in the finished product performance.

Despite the stated problems, our team focused in doing the best we could in the entrepreneurial part of the competition, we learned has much as possible about the portuguese health care system and we studied how to apply our product in a way that could benefit everyone. We talked with doctors, hospital administrators and patients, building with this information a business model that could fit all the needs from this different parts.

We did our best to spread the word regarding the SensUs competition and RA symptoms and population impact, we did presentations in our university, we joined social media platforms so that we could easily communicate with our colleagues.

Finally, we want to thank to the SensUs organization team that was always very helpfull and every time our team had a question or a problem it was easy to communicate with them and find a solution that worked best for the competition and for our team, and for that we are very gratefull. We also want to thank to all of the people that gave us support trough this process, Prof Hugo, Prof Catarina, Prof Manuel and all its team, Prof Guiomar and all the university staff that was involved in this project.

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