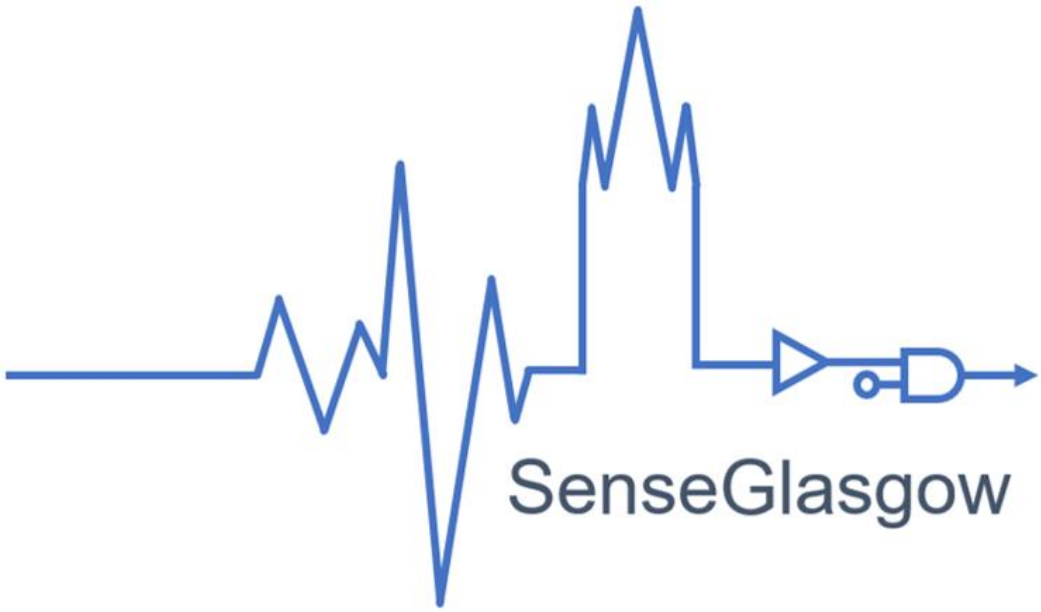

University of Glasgow

Team Members

Marycarmen Flores López

Mohammed Amin Benahzil

Daniel Vilafranca

Álvaro Pérez Guardiola

Alexandra Moreno

Ivona Ivanova

Temisan Atsegoh

Supervisor

Julien Reboud

Coach

Ioana Susnoschi-Luca

Date of submission

13/08/2020

Summary for the SensUs website

SenseGlasgow is developing a biosensor that is turning things around. The toxicity of VPA is linked how it is broken down in the liver of patients and SenseGlasgow is using that same process to measure the amount of VPA circulating in blood. To ensure the sensor only measures VPA and not other components present in the blood, the device uses antibodies specific to VPA tocapture the molecule on microbeads, before performing the measurement using an electrical signal. The electrical signal is generated by the reaction with a human P450 enzymeCYP2C9 that is immobilised on a metal electrode. The reaction is the same that happens in the body and generates 2 electrons. Instead of being lost in liver cells in the body, these electrons are stolen by the electrode, going into a read-out circuit. When The sensor is controlled by our phone app Valprosense, which makes monitoringValproate levels easier and faster, leading to reassured patients, leading more comfortable lives.

Biosensor system and assay

The principle for quantification of this biosensor is that the consumption of electrons by the P450 Enzyme CYP2C9 catalytic cycle is modulated by the available concentration of substrate when O_2 and electron saturation is achieved. VPA in the sample is immobilised in the sample chamber, and a separate known quantity of Valproate is used to indirectly measure the initial sample concentration through the reaction described above.

Molecular recognition and assay reagents

VPA molecules present in the sample can be captured anti-VPA monoclonal antibodies (Mabs) immobilised on protein G-coated beads. By using a magnet, beads are concentrated at the bottom of the chamber, and unbound molecules are washed away. Constant amount of stored VPA (sVPA) is added to bind to remaining antibody binding sites. Beads are concentrated at the bottom of the chamber, then unbound sVPA is pushed to the cytochrome chamber (Figure 1).

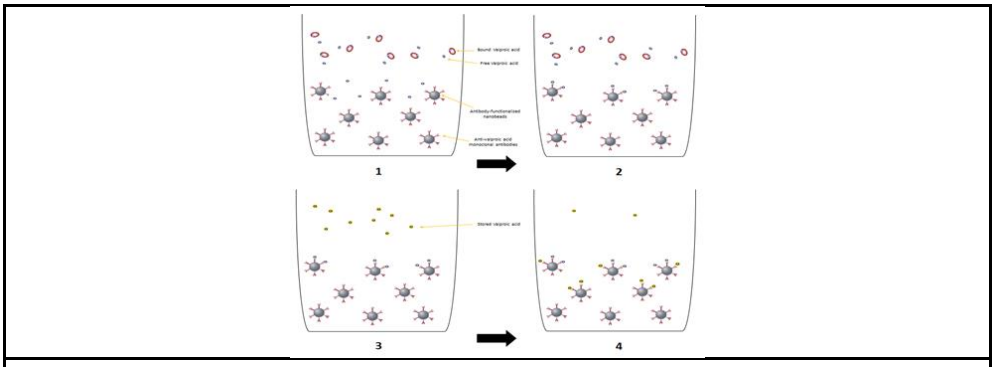

Fig. 1 – Working principle of the antibodies chamber.

Physical transduction

CYP2C9-Electrode Biosensor for Blood Dose Monitoring

Direct Electron Transfer and Michaelis-Menten Equation

Measuring the quantity of VPA via this enzyme reaction is possible with the direct electron transfer (DET) concept (proposed by Scheller *et al* as far back as 1977) utilising a gold (Au) electrode, as the Heme group from P450s can transfer electrons directly to and from an electrode surface (Schneider & Clark, 2013).

CYP2C9 Immobilisation to Au Electrode

The immobilisation technique proposed for this biosensor was pioneered by Gannett *et al* in 2006 and consists of covalently binding CYP2C9 to a self-assembled monolayer (SAM) of 11-mercaptoundecanoic acid (MUA), an organic acid with alkane-thiol tails, via a lysine residue in the enzyme's N-terminus. This methodology was chosen as it is largely uncomplicated and requires only the use of widely-available reagents and equipment, while still successfully overcoming both steric and orientation issues common to P450 enzyme immobilisation. This SAM is attached to the electrode surface with octane thiol according to methodology by Gannett *et al* (2006).

CYP2C9-MUA/OT-Au Immobilisation and Electrocatalysis

The chosen MUA-SAM immobilisation technique relies on a preferential binding of N-terminus lysine residues to MUA over protein-surface lysine residues. This preferential binding of the N-terminus may be due to the lower pK_a of the N-terminus lysine and greater steric constraints affecting the surface lysine residues. Orientation of the heme group was confirmed with X-ray photoelectron spectroscopy *(Gannett et al, 2006)(Yang et al, 2009).*

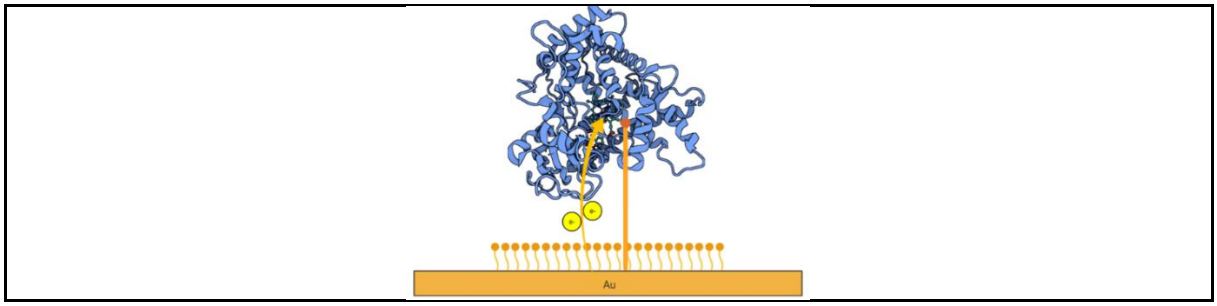

Fig. 2 – CYP2C9 bound to alkane-thiol branch of SAM on gold electrode. Heme group shown as stick-and-ball prosthetic group bound to SAM branch, creating a distance to electrode of 5.3nm. Two electrons transferred to Heme group shown

Cartridge technology

The cartridge can be seen in Figure 3. Its components are as follows:

Blisters: these are pressed on by actuators to push fluids through the chambers.

Sample chamber: the sample is inserted here. It contains antibodies that immobilise VPA molecules on the chamber surface. The waste substances from the sample are flushed into the waste chamber through the action on Blister 1.

Stored VPA chamber: this contains a known quantity of Valproate, which is pushed into the sample chamber through the action of Blister 1. This allows for the indirect measurement of the VPA concentration in the sample as described in the measuring process below.

Waste chamber: waste substances are flushed into this chamber through the action of Blister 1.

Reaction chamber: this chamber contains an enzyme-electrode complex that can measure the quantity of VPA present as described in the Measurement section. Non-immobilised VPA is pushed into this chamber through the action of Blister 2.

Flow gates: these are controlled by the actuators to direct the flow into the waste or reaction chambers.

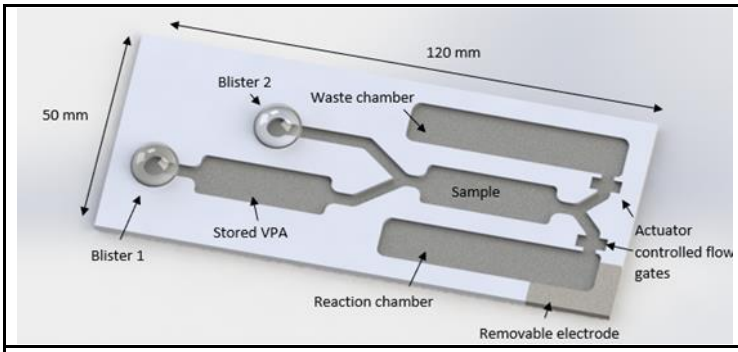

Fig. 3 – Diagram of the cartridge used in the design, showing the different chambers and components. Source: authors' own.

Reader instrument and user interaction

The reader consists of wires that are connected to the detection electrodes. The 3 electrodes are connected to a breadboard where the signal is filtered by resistors (one for each electrode) the output current is connected into the analogue to digital converter integrated into the Arduino which is coded to use the Bluetooth module hc-05 and send a message to the users device specifying the valproate levels translated from the current measured. The message is read and interpreted by a phone app: 'Valprosense' which adds automatically the concentration measured to the VPA level tracker functionality.

The idea is that the patient turns on the Arduino system, then connects the electrodes (connected to the Arduino) with the cartridge, then the user gets a message interpreted automatically by the phone app. The user may add the measured concentrations manually or by Bluetooth messaging and the app automatically keeps track of this levels alerting if some abnormality appears. The app also includes a functionality for finding support for epilepsy and bipolar disorders as well as contacts from people that have worked or are working with VPA. As well as information on nearby clinicians that may give the attention a patient may need. The user Interface and functionalities can be seen in Appendix B.

Technological feasibility

Due to their natural immunological function, Mabs are more selective than the other biorecognition elements nucleic acids/aptamers and MIPs, whose selectivity can be hindered by nonspecific electrostatic interactions and non-specific binding of analytes with similar structures (Morales and Halpern, 2018).

Microbeads allow the immobilisation of total number of Mabs molecules in the antibodies chamber (≈3cm^3). Based on the dimension of a Mab (14.5 nm × 8.5 nm × 4.0) nm (Tan et al., 2015), the surface area of each Mab molecule is 34 to 123.25 nm^2 depending on predominant orientation of Mabs (Figure 4).

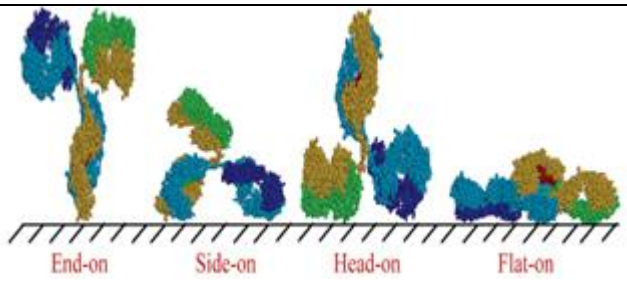

Fig. 4 – Possible orientations of Mabs molecules on the surface of solid supports (From Xu *et al*., 2006).

As for 1.25x10^{16} molecules of Mabs, total surface area is 42.5 – 154.06 x10^{16} nm2 or 42.5 – 154.06 x10^2 cm^2. Whereas the base of the antibodies chamber has a surface area of 3 cm^2.

By using magnetic beads of 1µm in diameter (or 3.14×106nm^2), 1.25x10^{16} molecules of Mabs can be immobilised on 1.04x10^{10} to 3.75x10^{10} beads, which is equivalent to a volume of 5.45x10^{18} to 19.65x10^{18} nm^3, 5.45 to 19.65 mm^3, or 5.45 to 19.65 µL.

In order to use 1.04x10^{10} beads only, which equates 5.45 µL, the orientation of Mabs must be predominantly end-on (**s**ee Figure 4).

Affinity-based immobilisation strategies allow an orientation-specific immobilization of Mabs on solid supports. Out of these, Fc region binding protein G can allow End-on orientation and increase Mabs loading on immobilisation surface much more efficiently (Figure 5) (Tsekenis et al., 2019; Makaraviciute and Ramanaviciene, 2013; Huy et al., 2011).

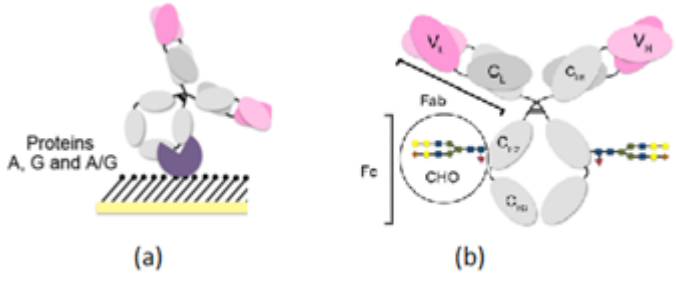

Fig. 5 – Immobilisation of Mabs on solid supports using proteins A and/or G.

Below is a detailed description of the measuring process within the cartridge:

1. The sample is inserted into the sample chamber via a one-way valve. This chamber contains the antibodies that detect and immobilise Valproate.
2. An actuator pushes on Blister 1 and closes the flow gate by the reaction chamber. This pushes the stored Valproate into the sample chamber, and the waste substances from the sample chamber into the waste chamber.

3. Some stored Valproate molecules are immobilised by any antibodies that are not already holding a Valproate molecule from the sample. Once the antibodies are saturated, the remaining Valproate can be used to determine the initial concentration in the sample.
4. An actuator pushes on Blister 2 and closes the flow gate by the waste chamber. This will push the non-immobilised Valproate into the reaction chamber, where the enzyme-electrode complex can quantify the amount of Valproate that was not trapped by the antibodies.
5. Since the number of antibodies and the concentration of the stored Valproate are known, the number of Valproate molecules in the sample can be calculated using the following equation:

$$n_{VPA\ (sample)} = n_{antibodies} - (n_{VPA\ (stored)} - n_{VPA(detected)})$$

Feasibility of Physical Transduction Method

The substrate concentration [S] can be measured via cyclic voltammetry (CV), where the current (*I*) in solution is measured while the voltage (V), or potential, of the electrode is increased to a maximal point – forward sweep – then decreased to the starting point– backwards sweep. **CV readings will show a peak of current at the electrode potential at which it is electrochemically favourable for electrons to transfer to the solution/enzyme**, known as the reduction, or cathodic peak. Given that this reduction is an electrochemically reversible reaction, electrons will flow back during the reverse sweep forming an oxidation, or anodic, peak. **Addition of reaction factors to the solution such as O_2 or warfarin will alter the solution so that it is more favourable for the electrons to transfer from the electrode,** thereby modulating the current at the reduction and oxidation peaks (Yuan *et al*, 2015). The resulting CV graph is shown in Fig. 12 with the effects of N_2 saturation, O_2 saturation and addition of warfarin.

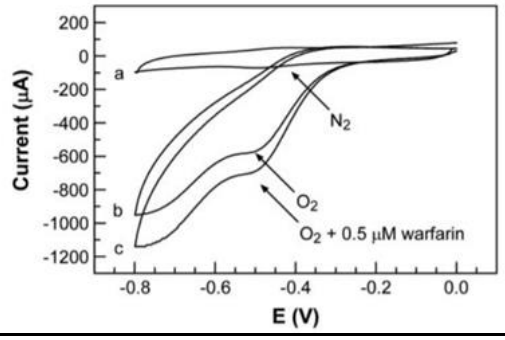

Fig. 6 – CV graph of the CYP2C9-SAM-Gold electrode in N_2 saturation, O_2 saturation and O_2 saturation + 0.5μM warfarin. (Yang et al, 2009)

If the cathodic peak current values are substituted into the Michaelis-Menten equation such that I_o (the current at the cathodic peak) is substituted for V_o and I_{max} (the maximum cathodic peak current at substrate saturation) is substituted for V_{max}, the equation then looks like this:

$$[S] = \frac{I_o \times K_M}{I_{max} - I_o}$$

The only required factor for determining [S] thus becomes K_M, which can be found by pre-testing with a range of substrate concentrations until max current is saturated and plotting the I_o of each cathodic peak vs the substrate concentration, as shown in *Fig. 13* by Yang *et al* (2009).

Originality

(1) A piece written by the Team;

SenseGlasgow had managed to create a biosensor based on the metabolism of VPA in the body to toxic compounds. The team had a long way while coming up with the right approaches about how to proceed with the given task and what will be the plan that they should they throughout the whole process, however, skills like hardworking, persistence and creativity that each member has, were the one that led to the successful reach of the final stages and finishing the project.

Even though the device is now fully designed, it is worth to point out the instances where the team has shown a highly creative ideas to overcome the problems. These go from creating new functionalities to expand the market size originally available to proving mathematically thee high probability of a resulting working device without access to labs or scientific instrumentation.

The team has developed an innovative way whereby means of using magnetic beads, an expansion of area occurs that allow antibodies to fit in such a small space like the one in our biosensor as well as studied how to immobilize the enzyme, its catalytic cycle and how to measure the changes provoked by a current change

The team did not put any limits to themselves during the process and this led to the creation of successful final product.

(2) A piece written independently by the Team's Supervisor/Professor.

SenseGlasgow's proposed approach to measure the concentration of valproate in serum is uniquely combining a (i) standard competitive assay scheme in an attempt to prepare the biosensor for the capability of differentiating bound valproate from the unbound form, with (ii) magnetic sample processing to increase specificity, and (iii) sensing using a metabolising enzymatic reaction, coupled to an electrode-based readout.

The uniqueness of the approach comes from the latter point, utilising a reaction happening in vivo (metabolising of VPA in the liver) as the sensing mechanism. This had been promoted in the early days of biosensing, but was deemed challenging due to issues with selectivity, as enzymes are often able to metabolise more than one compound.

It is worth noting that my role in the developments has been minimal and the team has carried out the whole project with really minimal support, from conception to iteration of the ideas and their implementation. This is evidenced in the selection of the idea, where the team started from the problem (valproate metabolism) and turned it around into their detection strategy.

I have been impressed by the creativity of the team and their ability to rebound from the hurdles they faced. For example, as the potential limitations in selectivity appeared in their research, they started building more functionalities in the biosensor, recognising its impact in differentiating bound/unbound forms of the analyte.

Finally, it is interesting to note that the Team also came up with a potential application in precision medicine: as the sensor uses the metabolising processes, it could be used to explore patients abilities to metabolise the drug, so not only monitoring and adjusting the dose, but also understanding the root cause of the problem for a more fundamental resolution.

Translation potential

Business model canvas

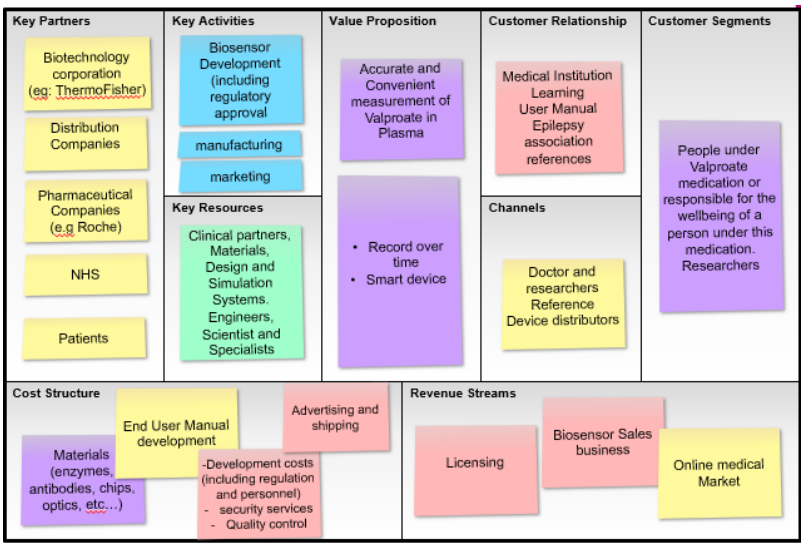

Fig 7. Business model Canvas

Stakeholder desirability

Epilepsy is one of the most common neurological diseases. Worldwide around 50 million people have epilepsy. In terms of the UK, 1 in every 100 people suffer from epilepsy (ca. 600,000), with around 30,000 newly diagnosed cases every year. Patients have very limited monitoring possibilities including: Therapeutic drug monitoring (i.e Valporate, Carbamazepine etc) and Invasive monitoring (250 cases/year). From these, 1/3 of patients are prescribed valproate, and must undergo at least 5 blood tests to monitor drug concentration in the blood. The results from these blood tests are available 24 to 36 hours, so patients are not able to monitor their blood frequently due to costs and waiting time of results.

Ideally, patients should be able to monitor the drug concentration in their blood in order to avoid hepatoxicity and have a better quality of life. Our biosensor would help solve these problems for patients prescribed valproate as not only would results be available in 15 minutes, but there would be a record of previous results making it possible to see the patient's evolution and more accessibility. Due to epilepsy being one of the most common neurological diseases this biosensor would be more convenient even for doctors and hospital management as the at home 24 hour accessibility of testing would reduce the amount of patients having to make appointments with doctors to conduct a blood test and would reduce the strain on the hospitals as less patients would be admitted for seizure relapses.

The biosensor would make it possible to only have to check in with the doctor if abnormalities in blood test are found, therefore making it a more efficient experience for both doctor and patient. Insurance companies would also benefit from the wide spread use of this biosensor by epilepsy patients as they would process less paper work as the 50 million people affected would be conducting at home testing, therefore, not billing the insurance company every time they do a blood test.

The product and services of the biosensor would be a VPA home based biosensor. The demand for this biosensor is based on the need to get an accurate prescription to control symptoms and limit to hospital visits. The lack of availability of this kind of biosensor creates for excessive medication causing patients to experience side effects and seizures without previous advice. Since currently blood tests to monitor the amount of drug concentration are not conducted with frequency patients can be prescribed high doses of medication due to the infrequent nature of testing and the lack of patient evolution track.

However, with this biosensor accurate awareness of the amount of AED in the blood would be available around the clock, a more patient personalized medicine, less risk of seizures and side effects of medication and less visits to the hospital. Therefore, the gain creators would-be 24-hour availability, faster results, and portable. The pain

relievers are at home usage, patient evolution track, General practitioner communication and smart device that records results on an application. Overall, this biosensor is a more convenient and patient friendly way of monitoring drug concentrations in the blood of epilepsy patients.

Business feasibility

The biosensor's development showcases 3 fundamental activities for the release of the biosensor into the marketplace: Development, Manufacture and Marketing.

The first activity implies the design thinking and simulation process done by engineers and biologists with the support of clinician's advice using various software such as Solidworks and MATLAB. The activity results in a cartridge scaled drawing with detailed specifications on assay preparation, reagents, chemistry reactions and physical transduction of the analogue signal into a digital signal shown in a reader which is also programmed to send a text to the user's phone app and keep a record of it. The regulatory approvals in the UK (i) the Medical Devices Regulations 2002 (SI 2002 No 618, as amended) and (ii) the General Product Safety Regulations 2005 (SI 2005 No 1803) are taken into account during this stage so that it fulfils all requirements to be in the market.

Manufacture, on the other hand, builds prototypes for performance and stress testing as well as mass production of the final biosensor. Aside from the materials used for the cartridge and biological surfaces which will be provided by a partner biotechnology company, it is necessary to have the equipment to transform and combine this materials into the final products (e.g. Laser cutters, centrifuge, incubator)which implies that a qualified user is also required to manipulate such devices (engineers, technicians, scientists). Patients play a key role during the testing of the software, as our main stakeholder, they participate in the black box testing of the device and the app. The final products are then processed by the NHS which check the requirements validation and packed to be distributed by a partner distribution company to pharmacies around UK.

Finally, the marketing activity was thought to be part of a licencing commercialization agreement with the key partners mentioned. The idea is to apply the strategy of commercialization shown by Servo in '*Licensing with Developmental Funds'* where our company acts as the technological firm selling a license to international distribution company that can potentially convince customers of the quality and reliability of the product (Servo, 1998). The licensing will be revenue dependent with a royalty percentage on agreement with the other company. however, is estimated a 10% royalty percentage as a suitable agreement.

Financial viability

The product's final cost was derived from the consideration of a cost-based price and market-based price. The former refer to the addition of all individual cost of each material and service used in the biosensor's development and the desired profit and the latter one to the price of other biosensors found in the market which could lie between 8£ (STANDARD Q COVID-19 Ag) up to 133£ (Freestyle libre (glucose)).

Considering that Drug monitoring occurs 3 to 5 times in a year (Pharmacist's Letter, 2010), it would be beneficial to give the product in 2 presentation. The first one would be the biosensor with a paid reader with a price of 25£ while the second is a pack of 3 biosensors with a free detector with a cost of 50£, the resulting profits are expected to be around 10% of the price of the presentation (2.5£ and 5£ accordingly)

As mentioned, a license agreement is the commercialization strategy chosen and thus, it is necessary to negotiate the license conditions and convince the partner that our biosensor will create a win-to-win situation for both parties involved. The challenge arises when the market size is considered, where the numbers presented above become unrealistic. Although it is true that 48k test could be sold if sold to all patients under VPA in the UK, it is also true that the regular monitoring of Valproate is not advised by the NICE (The National Institute for Health and Care Excellence, an institution in charge of providing guidance, advice and information in the healthcare sector) (Healt, 2016) ,in fact, a survey conducted to a population of 11 patients with epilepsy by the team show that only 2 female were controlling Phenytoin concentrations. Doctors confirm this suggestion by saying that hepatotoxicity is the only measure controlled and that some studies reject VPA for therapeutic drug monitoring as it does not provide substantial improvements. Reason why the team has found 3 alternatives to expand the UK based marketplace.

Internationalise the biosensor is one of them. Many non-developed countries do not possess the equipment necessary to conduct such test and when are requested by doctors, the test is sent abroad to be examined making the cost and results time to increase. Alternatively, a patient buying the 3 biosensors pack, would avoid unnecessary waste of money and time along the year. The second alternative is the detection of other drugs with the same device. Epilepsy drugs Phenobarbital is a substrate, and Phenytoin is both a substrate and an Inhibitor (which then induces function of the enzyme) and similar to these, there are more than 30 drugs that, in combination with the antibody chamber with the cartridge design, could be measured. Third and last alternative is to use it as a companion of diagnostic studies, the accessibility provided by this biosensor enables this for studies such as a VPA therapy guided by the patients P450 genotype & phenotype. Studies found patients with a low expressor phenotype or defective genotype in enzyme CYP2C9 can have as much as 2x the VPA blood levels from the same dose and 7x the risk for hepatotoxicity (Monostory, et al., 2019) (Zhao, et al., 2017)If VPA blood doses are adapted to these metabolisms, at-risk patients can benefit from VPA safely as lower doses will lead to equal therapeutic effect in patients with a lower P450 metabolic capacity (Budi et al, 2015). Due to the risks and ethics concerns of VPA therapy for at-risk patients, this therapy would be provided with extensive VPA blood monitoring which our biosensor can provide.

The solutions presented change the market size from an ideal maximum 48k to at least a million considering a global market perspective. This gives more freedom when designing the cost projections. Table 1 shows the expected revenues and sales we will likely obtain within the next 4-year period. They were calculated by estimating that the minimum budget required to develop 100 biosensor is around 600 £ just for the obtention of materials and that the process requires a team of at least 1 software engineers (2000£) , 2 scientists (4000£) and 2 engineers for the manufacture of the product (4000£), a minimum total budget for 3000 biosensors should be about 26700£ for a startup including the distribution and marketing expenses. Therefore, the Revenues start in -26700 and assuming during the first year the product needs more marketing or updates it could take up to -29500£. Using the profits we expect to make from each sell (2.5 for individual packages) a cost projection for the next 4 years can be made, it is shown in table 1 and figure 8. The latter figure continues after the 4th year expecting that the product follows a normal life cycle it is expected that reaches a stage of decline following the maturity stage.

Table 1: Cost projection for individual packages

	Sales (£)	Revenues (£)
2020	0	-29,500
2021	3,000/2 months	18,300-26,700= -11,200
2022	3,200/2 months	48,000-11,200= 36,800
2024	4,000/2 months	60,000+39,600=96,800
2025	6,000/2 months	90,000+99,600=186,800

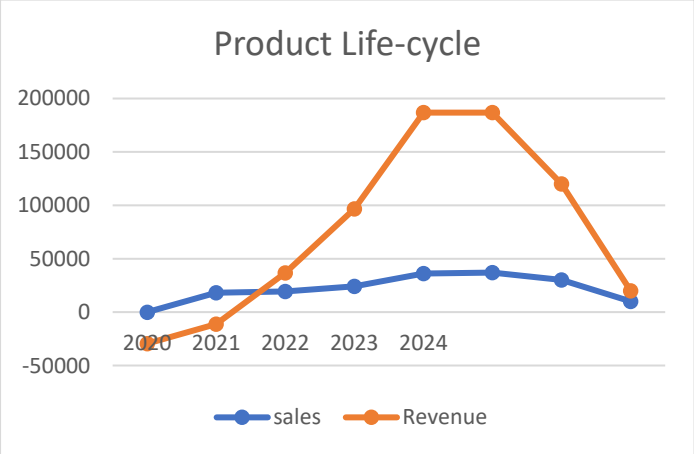

Fig. 8: Product life cycle for individual presentation

Team and support

Contributions of the Team Members

Marycarmen Flores López: Besides having designed the reader's user interface, was responsible of the entire Business model plan including entrepreneurship assignments, pitch, and interviews for model validation.

Amin Benz: Led, researched and designed the antibody isolation chamber. Co-authored the creativity pitch and Biosensor & Assay/Feasibility TRD sections. Supported Entrepreneurship Assignments.

Daniel Vilafranca: Led, researched and designed the electrode transduction chamber. Co-authored the creativity pitch and Biosensor & Assay/Feasibility TRD sections.

Álvaro Pérez Guardiola: Was one of the team leaders and focused on cartridge technology and design, as well as general group coordination and liaison with SensUs. In addition, designed the team logo and made the team's 1-minute pitch.

Alexandra Moreno: Contributed to the team by conducting research for scientific papers that would support the feasibility of our biosensor working. Also, created the team banner and oversaw the stakeholder desirability of the TRD

Ivona Ivanova: Contributed to the team by helping with electrode reusability and assisted with the research for creating electrode system. Wrote Originallity and Summary TRD sections

Temisan Atsegoh: Assisted in research and writing up.

People who have given support

Zulun Ye (University of Glasgow, Scotland): MSc student in electronics & electrical engineering, Advisor for the electronic set up of the device.

Josefina Otero (Universidad de la Republica, Uruguay): 4th year accountancy student. Collaboration in cost modelling and projection terminologies and calculations.

Sponsors

Nucleo de Ingenieria Biomedica (NIB) Universidad de la Republica, Uruguay : Live guidance with the main head of the department: Franco Simini for the detection system.

Final Remarks

It should be noted that the COVID-19 crisis affected the SenseGlasgow team particularly hard, as the team members come from five different countries across three continents. This proved to be a challenge initially, as different time zones and Internet access had to be considered when scheduling meetings.

In addition, the team's inability to access laboratories limited the scope of this project's possibilities significantly: no physical testing was performed due to this limitation. This is something that the team is hoping to be able to expand on in the future once access to labs is granted.

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Appendix A

Raw cost list of materials. -100 biosensors with detection system

Material	Price (£)
PMMA (cartridge material)	15
Silver Electrodes	20
Gold Electrodes	30
Platinum Electrodes	30
Piranha Solution	100
De- ionised H2O	1
Ethanol	1
NaCl	1
PBS	1
8-octanethiol	50
11-mercaptoundecanoic acid	70
Flurbiprofen	33
Dapasone	36
Arduino nano	30
Breadboard and accessories	40

Appendix B