BiosensUM

Team Results

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BiosensUM

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1 - Summary

This year, BiosensUM was given the challenge to detect and quantify Interleukin-6 (IL-6), a major inflammatory cytokine responsible for the induction of many immune responses. IL-6 is an essential biomarker of sepsis and septic shock, which causes the death of over 5.3 million people annually.[1] Our biosensor uses localized surface plasmon resonance (LSPR) technology on borosilicate tapered fibers (TBF) to detect the IL-6 concentration of a 20 µL **plasma** sample within a range of 1 to 2,000 pg/ml in 5 minutes. The lack of conclusive diagnostic tools for sepsis and the real-world relevance and international aspect of this project drove and motivated our team to develop an innovative diagnostic device. Since cytokines exert biological effects at low doses, our biosensor is designed to rapidly detect circulating levels of IL-6 for real-time monitoring of acute inflammations. BiosensUM's main goal this year is to promote scientific innovation for better healthcare worldwide. Our easy-to-use POC technology would be one of the first biosensors to introduce reusable cartridges. We are also eager to share our ideas with the other teams during the innovation days. We truly believe that through competitions that promote innovation and learning, we can have a beneficial impact worldwide!

2 - Biosensor system and assay

Interleukin 6 (IL-6) is one of the numerous biomarkers that can be used to diagnose an inflammatory disease.[1] Sepsis is defined as life-threatening organ dysfunction caused by dysregulated host response to infection[2]. Despite existing diagnostic criteria, early diagnosis of sepsis is usually complex due to its heterogeneity which makes it frequently misdiagnosed. Sepsis cases are diagnosed either through laboratory analyses such as blood tests measuring for different inflammatory biomarkers or through symptomatic assessment such as the quick Sepsis Related Organ Failure Assessment (qSOFA).[3-5] IL-6 is a pleiotropic proinflammatory cytokine and a major regulator of the inflammatory cascade in the immune system.[6,7] It is produced by activated macrophage and other immune cells such as non-lymphoid cells, adipocytes and astrocytes and it is responsible for lymphocyte activation and regulation of B- and T-cell functions such as the production of antibodies and the induction of systemic reactions (with IL-1ß and TNF-α, amongst others) such as fever, acute-phase inflammation and, in some cases, [septic] shock. Previous studies have shown that plasma levels of cytokines in septic shocks correlate with disease severity [8,9], that cytokine levels are more stable in plasma than in serum, where there is more non specific background[10-12]; but also that IL-6 is a prognostic indicator of outcome in acute inflammation[13] as well as an accurate biomarker to identify sepsis in plasma samples [5]. Therefore, our proposed biosensor needs to rapidly quantify the IL-6 concentration in order to diagnose and monitor sepsis cases, as well as other inflammatory states[5]. Thus, we designed a biosensor based on LSPR technology combined with an immunological assay on sharp tapered fibers that allows for a rapid quantification of IL-6 in plasma.

2.1 Molecular recognition and assay reagents

The first step in the detection of IL-6 is to bind the protein unto our TBF. IL-6's specific binding was accomplished using monoclonal antibodies (mAb: L-519, L-395, Hytest) immobilized on gold nanoparticle-coated TBF (Shutter Instruments). The 10 cm length borosilicate fibers were pulled using a Shutter Instruments P-2000 Pipet Puller to about 200 nm tips (Appendix 1).

These fibers greatly increase LSPR signal by promoting interaction between the light and the gold nanoparticle coated surface. The fibers are coated with homemade 40 nm gold nanoparticles (AuNPs). The AuNPs were synthesized according to Bastús et al.'s protocol.[14] The 40 nm size was confirmed by UV-Vis Spectrometry. The UV-Vis measurement showed a plasmon peak at 530 nm, which confirms the size of the AuNPs, since the size of the NP influences the peak position (Appendix 2). The nanoparticle-coated surfaces are prepared by dipping the cleaned fibers in a di-block polymer of polystyrene and poly(4-vinylpyridine) (PS-b-P4VP) (see appendix 3).

The positively charged pyridine group of the P4VP forms an electrostatic bond with the negatively charged citrate capped AuNPs. Once the AuNPs are on the fibers, we conjugated the detection antibodies onto the fibers by electrostatic interactions. Briefly, the fibers were dipped in a 20 ug/mL antibody solution for an hour on an orbital shaker at 100 rpm, then 20 uL of a 1mM $^{-}$OOC-PEG-SH solution was added and left on the orbital shaker for an additional 30 minutes.

2.2 Physical transduction

The LSPR is an optical phenomenon generated by collective oscillations of the electron gas in metal nanostructures surrounded by a dielectric.[15] Typical materials for plasmonic applications are noble metals, especially silver or gold. Despite silver displaying sharper and more intense LSPR bands than gold, gold nanostructures have a higher stability for biosensing applications.[15,16] This method relies on a simple optical system : when metal nanostructures interact with a light beam, part of the incident photons are absorbed or scattered. Both absorption and scattering are greatly enhanced when the LSPR is excited. The interaction of gold nanoparticles with light is strongly dictated by their environment, size and shape or refractive index.[17] Indeed, the oscillating electric fields of a light ray propagating near a nanoparticle interact with the free electrons causing an oscillation of the electronic charge which is in resonance with the frequency of visible light. These resonant oscillations are the surface plasmons. For small gold nanoparticles of approximately 40 nm, the surface plasmonic resonance causes light to be absorbed in the green part of the spectrum, around 530 nm[14], while the complementary color of green is reflected, thus giving a red-magenta color to the solution.

The intense electromagnetic fields induced by the LSPR provide a sensitive probe to detect small changes in the dielectric environment around the nanoparticles. When IL-6 proteins bind to their corresponding antibodies, the mass bound to the surface changes, causing a red shift on the absorbance peak, which allows us to monitor variations

induced by biomolecular interactions at the surface of the nanoparticles via the LSPR peak shifts. This will create an area where the electric field is high and of about 70 nm[18] that can be affected by its environment, meaning that binding of molecules with the help of a selective receptor will affect it. When binding occurs, a change in the refractive index is observed, since the particle will absorb light differently. Since our biological elements are small, they will induce a change in the measurements.

2.3 Cartridge Technology

As shown in the figure below, our biosensor uses two white LEDs run by a microcontroller to analyse the plasma sample contained in the cartridge (See Appendix 4). The disposable receiver is a P20 micropipette tip sealed by melting its opening to make it impermeable. The metallic fiber connector is used to insert the TBF. The light is directed through optical fibers towards the alignment plate which aligns the optical fibers with the TBF that are coated with anti-IL-6 conjugated spherical gold nanoparticles. The signal is then brought back to the biosensor and digitized by a spectrometer also ran by the microcontroller. If IL-6 proteins are present in the sample, the intensity peak at the resonance plasmon wavelength will shift proportionally to the concentration of IL-6. This concentration is then determined by analyzing the spectrum of light reflected at the tip of the fiber. This system ensures no cross-contamination since the TBF are single use.

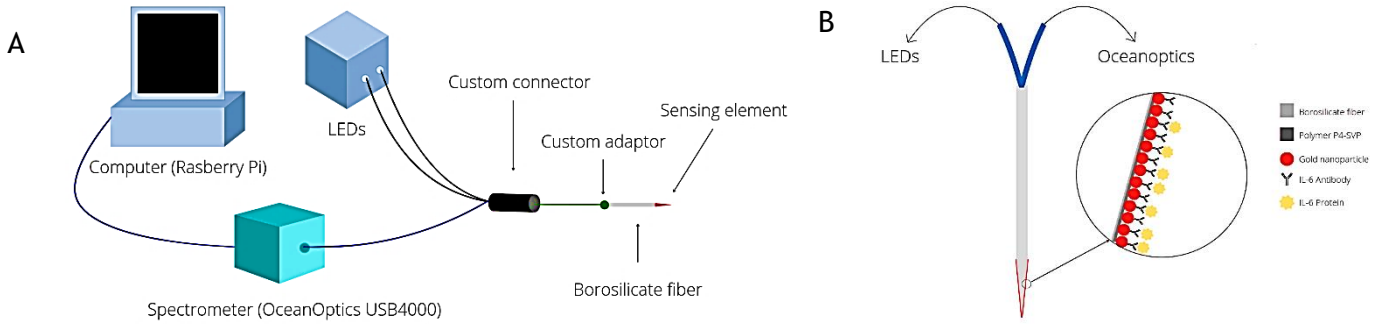

Figure 1. A) Design of the biosensing system and main components and B) scheme of a borosilicate fiber (TBF) functionalized with 40 nm spherical gold nanoparticles conjugated to the detection IL-6 antibody (L-519).

2.4 Reader instrument and user interaction

The reader instrument can be divided into two main categories: the hardware and the software. Our goal has been to integrate one with the other as best as we could to provide the best experience to the user. This explains why we chose to integrate a Raspberry Pi and a touchscreen since it is a powerful tiny computer that can be used very easily with our custom interface. To start a test, the only requirement is a touch input from the user on the touchscreen.

For the hardware, the user must interact with our custom connector to mount a new TBF for each test and must put the sample in our sample holder. This part will be optimized to have a time-efficient straightforward process. To make sure this process is well done, we will include a training section in our interface that shows exactly how to perform the manipulation. Once the analysis is completed, the touchscreen shows results to the user who can then use this information to act. The twofold port only needs three manipulations (see cartridge mechanism in 2.3), to insert (or change) the disposable FO sample in the slider, to insert the disposable receiver with its aliquot in the cartridge encasing and then to lower the slider. The hardware behind all of this contains the optical setup and the electronics. Our light source are two white commercial LEDs that connect with the FO with a custom-built adapter that we made, and it sends the light to our custom connector. For the electronics, we built a modular setup in which the received light goes successively through a portable spectrometer (Ocean Optics USB4000) which digitizes the data and sends it to a Raspberry Pi that computes the signal and based on our calibration curve, outputs the concentration that can then finally be displayed on the touchscreen. The Raspberry Pi runs a Python code (further described in 3.2.3) that gives a live feed of the received light intensity in function of wavelength and processes the data to lower the noise and give a precise measurement of the concentration. All our hardware, including the 7-inch touchscreen are inserted in a 3D printed case which dimensions are 19 cm x 21 cm x 10cm. The user ultimately must only insert 20 µL of plasma in the disposable sample container in the encasing, insert the disposable TBF in the sample and press start on the instrument. The machine then processes for four minutes before showing results. Once the results are shown, the user must dispose of the TBF sample and the aliquot in a biohazard and sharps waste.

Technological feasibility

This section presents the results of the most important tests along the remaining challenges and future upgrades and plans for the coming development of a fully market-ready biosensor.

3.1 Molecular recognition

First, the binding between antibodies and IL-6 proteins was verified by SPR with an Affinité Instruments P4SPR. The primary detection with only capture antibodies (L519) did not reach below 500 pg/mL, unfortunately. As for secondary detection with the capture-detection (L519 – L395) antibodies, it showed improvement but was still not enough to reach concentrations below 100 pg/ml. Therefore, we did a secondary detection with the detection antibodies conjugated to 40 nm gold nanoparticles (L519 – L359@AuNPs). The experiments were realized with IL-6 proteins in running buffer (RB) which was PBST 1X at pH=8.5 and the RB was used as a negative control to verify the specificity (Table 1).The tests showed that they bind undoubtedly well, since the SPR shifts were around 2500 RU and had about 7-12% non-specific interactions.

Table 1. SPR shifts for fixed IL-6 protein concentrations of 100 pg/mL in PBST with 10 µg/ml mAb L-519 immobilized.

Time (seconds)		Shift (RU)					Non-specific interactions (%)
Start	End	A Canal	B Canal	C Canal	Reference	Average	
4133.2	5592.5	2835.7	2947.2	2890.7	195.2	2891.2	7.2
7747.0	8876.4	1882.7	2167.1	2220.0	224.2	2089.9	12.0
9578.9	10509.0	2356.2	2467.3	2565.0	238.7	2462.8	10.7

3.2 Physical transduction

The physical transduction is a key portion of signal processing in all point of care devices. In our case, it can be divided into two main categories: the optical setup that includes the TBF where the LSPR reaction occurs, and the spectrometer and the software that analyses this data to quantify the concentration.

3.2.1 The optical setup

Our optical setup has been designed to maximize accuracy and simplicity. It includes LEDs, optical fibers, a spectrometer, and a custom connector. The figure shown in Appendix 5 presents our optical setup and its components. It also includes a study on the effect of light intensity from which we concluded that the higher the light intensity, the better the signal which is why we use two LEDs in distinct fibers to send more light to the sample.

The main component of our setup is the custom optical connector made from a modified syringe that allows us to change the TBF easily after mounting them in a syringe tip. The light is emitted by the LEDs, it travels to the connector and in the TBF where there is an LSPR reaction and it travels back to our spectrometer, the Ocean Optics USB4000, which is used to digitize the signal. For light to be propagated throughout the TBF, the LEDs must be aligned properly. It is essential that light travels in the fiber with an angle greater than the critical angle to achieve total internal reflection to the tip. This critical angle depends on the material of the fiber and the nature of the solution since it is related to the refractive index (see equation 1). The absorbance of light will depend on the concentration of IL-6 and of the coating of the fiber. Therefore, the structure of the tip will permit light to be reflected up the tip towards the detection elements.

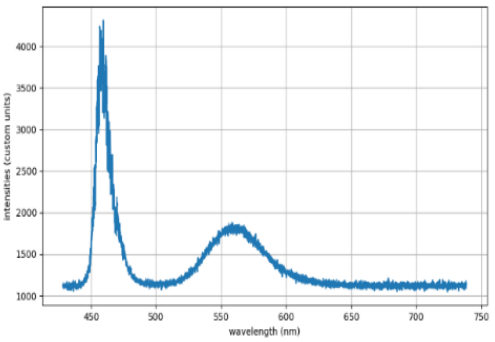

Figure 1. LED light spectrum with bare fiber.

$$sin\theta_c = \frac{n_2}{n_1} \quad (1)$$

Our setup was first validated by capturing the light spectrum of our LEDs using a non-coated TBF where we can observe peaks a 460 nm and 560 nm as expected. [19]

3.2.2 LSPR on tapered borosilicate fibers (TBF)

To detect interleukin 6, we used an innovative technology: LSPR on TBF. To achieve this, TBF are pulled using a specific protocol that creates a tapered tip that has been optimized to reflect light back to a detector. The tip can be used for LSPR reaction by coating it to provide the signal needed to quantify the concentration of the solution. We chose LSPR over propagative SPR since it has shown to be a very good option for quick and simple instrumentation biosensors.[20,21]

To confirm the binding of the AuNPs to the surface of the TBF, we compared the absorbance spectrum of a non-coated and a coated TBF. To the right are the results of this experiment where we can see a small increase of light absorption at around 500 nm that indicate the bounding of the AuNPs to the surface. We also took SEM images of our fibers (see appendix 1)

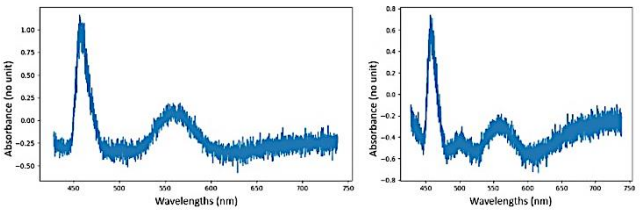

Figure 2. Absorption of non-coated (left) and coated (right) TBF.

3.2.3 Digital processing and calibration

Calibration establishes the link between the analytical response and the concentration of analyte present in solution by testing the device using standards whose exact value is known. Since the microfluidic cartridges were not ready for the calibration, we used an alternative method where the TBF are directly dipped in a P20 micropipette sealed tip containing the sample. To collect the signal, we used seabreeze which is a Python package developed to read data from an OceanOptics spectrometer. This library grandly facilitated the integration of the spectrometer with our device and with our custom software. [22] We included the main Python functions we used and included a link to all the code which we decided to open-source to give back to the scientific community in Appendix 7.

To quantify the concentration of the solution, we need to measure the wavelength shift of the most absorbed wavelength. This shift can be detected by comparing the spectrum of the reflected light after sample injection to the one prior to sample injection. First, to find the absorption peak at a given time, the spectrum taken is divided by a reference spectrum of the LEDs. During the measurement, the ratio between the measurement spectra and the reference gives the absorbance peak. By taking two spectra at t=0 in a buffer solution and t=4 minutes in the sample of a known concentration, it is possible to detect an absorbance wavelength shift and by repeating the process with several known concentrations we obtain a calibration curve that gives a correlation between the shifted absorbance wavelength and the sample IL-6 concentration. Thus, the concentration of any plasma sample can be determined by comparing the absorbance wavelength at t=4 minutes with the calibration curve.

After that, we use a Savitzky-Golay digital filter to reduce the noise in the signal that greatly improved the quality of our results. This filter is used to reduce the noise of a signal while minimizing the distortion. To achieve this, it makes use of a convolution process that fits subsets of points with a low degree polynomial to reduce the noise.[23] This technique is widely used in biological signal treatment like EEG but its use with LSPR optical detection is limited which shows that digital treatment of this type of signal represents an area of innovation we wish to further explore.[24]

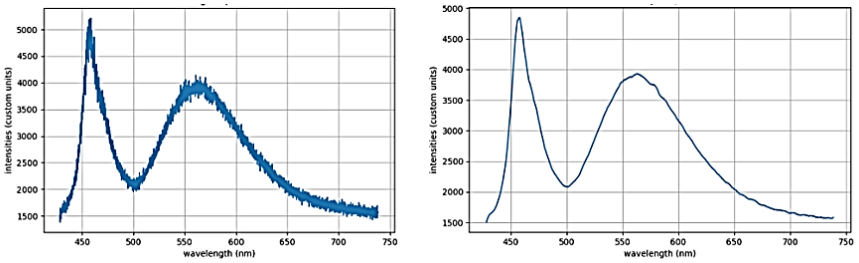

Figure 3. Comparison between the raw (left) and filtered (right) light spectrum.

The figure to the right shows the improvements we were able to achieve in noise reduction with a third degree Savitzky-Golay filter with an integration window of 105. After this filtering, we can extract the absorbance spectrum by taking the logarithm of the ratio between the reference and the measurement spectra as described in the literature.[25]

From this new curve and since we know that the peak absorption of our coating occurs at around 500 nm we can compute the position of the max absorption which is a function of the concentration of the solution. Using this method with multiple concentrations, we can extract a calibration curve with the concentration of the sample on the x axis and the position of the absorption peak on the y axis.

For our curve, we used concentrations of 55 pg/mL, 150 pg/mL, 350 pg/mL, 600 pg/mL, 1000 pg/mL and 1500 pg/mL. For each concentration, triplicates were taken to ensure accuracy and precision. The relative standard deviations (RSD) and confidence interval (IC) are respectively under 2 nm and 5 nm. A compliance ratio (CR) "Ratio de conformité") was also calculated for the lowest concentration, 84.4. A CR greater than 10 indicates that the actual detection limit of the method is lower than the detection limit estimated during the tests. The resulting curve can be observed below. We included the detailed results with the standard deviation in a table in Appendix 6.

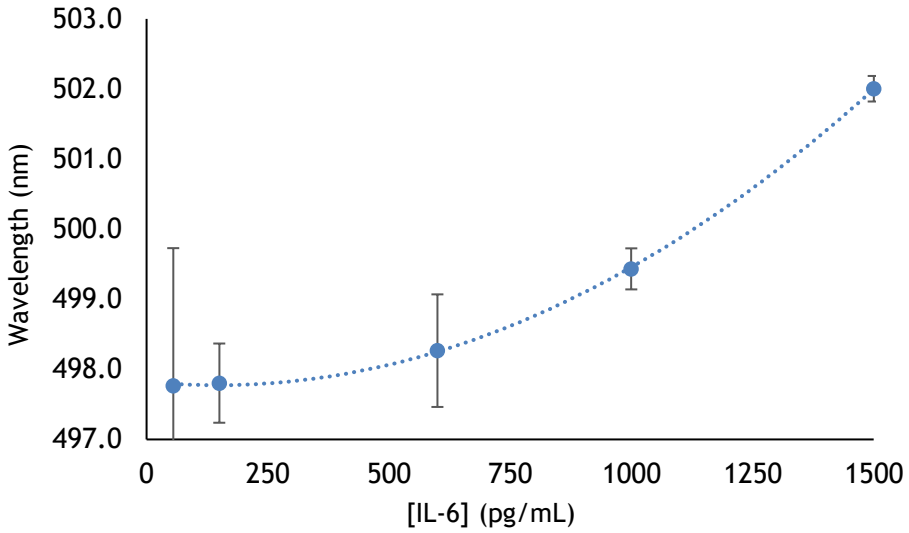

Figure 5. Wavelength calibration curve as a function of IL-6 concentration in pg/mL in PBST pH 8.5, where Wavelength = 2E-06 $[IL\text{-}6]^2$ + 0.0007 [IL-6] + 497.82 and R^2 = 0.9998.

3.3 Fluidic cartridge

Our fluidic cartridges are one of the main areas we would like to improve in the future. Because of the TBF, extra care was required to ensure their physical integrity which prevented us from using a PDMS cartridge to handle the sample which is why we compromised on ease of use to ensure the proper working of our design. For the tests, we used sealed-off P20 micropipette tips leak and then, using a custom support, we dipped the TBF in the micropipette tip to collect the signal. In a future version, we would like to have the pulled fiber tip integrated in a microfluidic device to minimize the manipulations required. We have an idea of what the design would look like (Appendix 4) but we did not have the time to implement it.

3.4 Reader instrument

The building of the instrument was an important part of the process as it is the bridge between the “technology” and the user. Our instrument can be divided in three main parts: first, the portion where the reaction will occur which is composed of the test stand and the optical fibers, second, the touchscreen which will allow users to interact with the device and third, the case that houses the components.

For the whole device, 3D printing was the way to go to develop an object that can be modified and built quickly. To make measurements, it is imperative to have a visual on what is measured by the spectrometer. For that part, the Raspberry Pi microcontroller paired with its touchscreen came in handy. We programmed them in such a way that together they compile the information, interpret them, and display them for the calibration and the testing process. That leaves the product with an easy to access interactive platform.

The reader instrument gave us great challenges through the integration of the optical setup and the coordination between the software and the experiments. To make it all work, the Raspberry Pi which is a portable minicomputer was used to power our device and greatly helped with this problem. The most challenging part is the optical alignment between the TBF and the optical connector. Since we change the tip for every test, it must be a non-permanent connection that is highly reliable which is why we built our custom connector out of a syringe and used syringe tips to house the TBF.

Overall, even though there are many areas to improve upon regarding our instrument, we are still very pleased with the result and confident about the methods we used to mitigate the biggest issues we encountered.

4- Originality

Team

The rapid detection of IL-6 in an hospital setting could greatly improve the diagnosis of sepsis and prevent deaths annually by allowing doctors to make the right decision faster. With that in mind, our team researched the literature on the subject and identified a few potential solutions that could be used. Our first option was a paper-based method that had the potential to be both very cheap and very accessible but after a lot of testing, we were never able to achieve the results observed in the articles we consulted. Therefor, we had to quickly redirect our efforts towards LSPR to detect the concentration of IL-6 in blood plasma since it is one of the specialties of our supervisor's lab and we had team members that were familiar with the technology.

The main aspect our team innovated in was on the surface chemistry of our LSPR-based device: we used a TBF that could be dipped in the sample to obtain a measurement. The optical path had been previously optimized to make sure the light travels back to the detector. The entire optical setup that allows us to use this technology and its integration with the pulled fiber and the spectrometer was designed and validated by the team.

Our goal was also to maximize ease of use and accessibility which is why we decided to use a Raspberry Pi computer with a touchscreen to facilitate its use. By doing so, it would allow our device to be powered with batteries and used in remote or rural areas that often don't have access to this type of technology. Since the Raspberry Pi is a computer, we could also add features like automatic reporting via email or data analysis on the cloud.

Supervisor

I confirm the statement from the students. The ideas presented in their document comes from their own work and resourcefulness. The team has been highly independent this year. I did a few 1-hour lectures at the beginning of the year to provide them basic information on sensing and on the task at hand, but since then, they have been highly independent. They have come up with their ideas independently. The main innovation this year is the use of tapered fibers, which they use to optimize the reflection of the light back to the detector. We are using similar tapered fibers in my lab for SERS measurement near cells, but I did not think that it would serve to increase light collection in fiber optic LSPR. This is also absent in the literature to the best of my knowledge. As such, this is an independent idea on their own. Regarding the other aspects of the competition, they have been self-reliant to write the team result document and for all the milestones in the course of this year. They have used resources from my laboratory. Among them, they validated their protein selection by using our SPR instrument. I do have two students working on LSPR but using dark field and one on detecting inflammation markers (not with LSPR). They surely have discussed with them, but the team's results are significantly apart from my own. No one in my group is doing LSPR on fiber optics. As such, I confirm the team's independent thought process.

My involvement with the team was limited to steering meetings on a monthly basis. Due to my sabbatical in the UK, nearly all interactions with them were online. As such, they worked remotely for the most part of their project, limiting the interactions with my team members, further strengthening their independence from my own work.

Pr. Jean-François Masson

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Translation potential

Business model canvas

<table>
<tr>
<td><u>Problem(s)</u>
• There is no specific diagnostic method or biomarker used for sepsis
• Time for sepsis diagnosis > patient deterioration often leading to multiple organ failure or death
• Cytokines exert effect at very low circulating concentrations</td>
<td><u>Solution(s)</u>
• IL-6 is a reliable and specific biomarker for early sepsis detection and evolution (prognosis)
• We offer a POCT capable of detecting from 1-2,000 pg/ml of IL-6 in plasma, which is representative of different inflammatory states</td>
<td rowspan="2"><u>Unique value proposition</u>
BiosensUM offers a POCT that can bring standardization in the diagnosis and prognosis (disease maintenance) of acute inflammations such as sepsis and septic shocks by measuring a specific biomarker, IL-6, which is also a major regulator of inflammation. Our TBF immunoassay technology has never been marketed before in the biosensing field. [26]</td>
<td><u>Unfair advantage</u>
• Team members who have participated in previous SensUs editions
• Team members who are already familiar with parts of our technology
• Access to professionals in parts of the technology used</td>
<td rowspan="2"><u>Customer segments</u>
• Early adopters (B2B2C)
Prescribers in hospital/emergency settings (clinical nurses and doctors)

R&D incubators and investors (such as pharmaceuticals for B2B2C

• In the future
Direct sales (B2C) to patients i.e., for chronic inflammatory diseases maintenance</td>
</tr>
<tr>
<td><u>Existing alternatives</u>
• Other team's biosensors (IL-6 detection)
• Routine tests on blood/urine (inflammatory markers, cultures)</td>
<td><u>Key metrics</u>
• Sales revenue
• Customer acquisition costs
• Customer churn
• Customer engagement
• Customer satisfaction [27]</td>
<td><u>Channels</u>
• PR, word of mouth, involving key partners (Canadian Sepsis Foundation, Ministry of Health and Social Services, R&D incubators, scientific conventions…)</td>
</tr>
<tr>
<td colspan="3"><u>Cost structure</u>
• Fixed costs
Labor costs (offshoring could bring the costs down)
R&D costs
Licensing and regulations (ISO), laboratory maintenance costs
• Variable costs
Raw material costs
Sample preparation innovation</td>
<td colspan="3"><u>Revenue streams</u>
Health insurance → compensation per test → laboratory
→ compensation per consult → outpatient clinic*
Outpatient clinic → lab test cost → laboratory
Customer's POCT use → ICT company reliability insurance → increases the number of buyers/users
* In the early years, we would like to extend our POCT to a usage in disease maintenance as well (i.e., chronic autoinflammatory diseases; cancers, COVID-19, arthritis, etc.). [28]</td>
</tr>
</table>

Market description

Nowadays, biological detection methods are ubiquitous in medicine and especially in diagnosis and prognosis. The biosensing market is mainly driven by scientific and technological advancements, especially since the emergence of nano- and micro-technologies. It provides solutions to design simple point-of-care technologies that can be used in any setting as they do not require scientific expertise for certain manipulations or data treatment.

The North American (NA) sepsis diagnostic market was valued at more than 430M$ USD in 2022 at a compound annual growth rate (CAGR) of 8,43% [29]; depending on the forecast period analyzed [30-33], the CAGR of this biosensing market is expected to go up to 9,8% by 2026,[34] which makes it an interesting industry to invest in. The sepsis diagnostic market has also considerably grown both in size and in interest since the COVID-19 pandemic, whereas the most common complications associated with this infection were related to acute and chronic inflammation.

BiosensUM offers a small, portable, and easy-to-use point-of-care technology that is simple to manufacture and allows for a label-free detection of a major regulator of inflammation, interleukin-6 (IL-6). The need for cytokine sensors has been growing and is especially noticeable in the case of inflammatory reactions, as the biomarkers currently targeted in biochemical analysis (see Appendix 8 are upregulated by inflammatory cytokines, which would make it more efficient to directly target these molecules.

Stakeholder desirability

After interviewing health professionals working in different settings (hospitals, pharmacies, laboratories, etc.), we have concluded that the main actors in need of our proposed technology would be the professionals working in a hospital setting, since sepsis cases and acute inflammations are treated in emergencies. Additionally, hospital administrations (HA) tend to order their equipment based on their professionals' recommendation and needs; HAs also input new diagnostic protocols based on the public health's recommendations; however, prognosis and maintenance protocols are established internally. Since there is currently no conclusive test to specifically diagnose sepsis, hospital laboratories don't have a standardized protocol or biomarker when diagnosing or monitoring acute inflammations. After focussing our interviews with hospital biochemists working on inflammatory diseases, we have learned that the main targets for sepsis diagnosis were two proteins: procalcitonin (PCT) and C-reactive protein (CRP). Since the levels of PCT considerably increase during bacterial infections, it is also useful in the management of antibacterial therapies. Nevertheless, PCT levels don't increase during viral infections and cannot give information on viral-related inflammatory reactions, which are, as demonstrated by the COVID-19 pandemic, also an important cause of chronic inflammation.

According to experts in immunology, since cytokines are the first molecules produced to initiate the inflammatory cascade, and thus the synthesis of PCT and CRP, directly measuring those targets would allow to predict the outcome of inflammatory reactions, by identifying physiological changes before the apparition of symptoms or complications. IL-6 has also been shown to be an accurate biomarker for the detection of sepsis or early sepsis and is a sensitive and specific target for the detection of sepsis or early sepsis.

In Canada, the health system requires a patient to have a lab test requisition form in order for a blood work to be done by a laboratory. Considering the fact that a blood test can cost anywhere between 150 to 3,000 $ CAD and can take from 1 to 24 hours depending on the test performed, offering a 5-minute alternative that costs less than 5$ per cartridge to produce (see Appendix 9) can substantially reduce the costs of sepsis. In Canada alone, there are approximately 75,000 cases of severe sepsis annually, and, if not treated quickly enough, they can lead to multiple organ failure and even death: it is also the cause of 1 in 5 deaths worldwide.

Although cytokine quantification has shown great potential in the management of inflammatory reactions, only a few laboratories actually have the equipment to perform such testing. In Quebec, for example, IL-6 quantification is classified as a superregional analysis, which means only two laboratories in the province have the means to receive and test samples for IL-6 quantification. For people whose healthcare access is already difficult, the need of a portable device can be a matter of life or death. The introduction of our biosensor in the healthcare industry has great potential for stakeholders as it presents a simple and effective solution to current diagnostic takebacks: by restricting the detection to a biomarker that has been shown to have specific diagnostic and prognostic value for sepsis and septic shocks, we are offering a cheaper and faster alternative to blood tests that would help mitigate sepsis mortality and morbidity by allowing professionals for real-time monitoring of acute inflammations. The proposed biosensor gives a quantitative result of the IL-6 concentration as well as a qualitative result with an easy-to-understand color code based on known clinical values of IL-6 (see Appendix 10).[35,36] Our new lab-on-a-tip (see Figure 1 and Appendix 3) design also requires a smaller blood sample volume (20 µL), which makes it less invasive than a regular blood test (approximately 10 mL) and no specific manipulation is required from the user, which protects them from any biohazard. Although BiosensUM is offering a POCT initially directed at prescribers in emergency settings because they are the first group of health professionals in need of it, our biosensor could also be used in different settings thanks to its potential of multiplexing for more than one inflammatory biomarker, such as in outpatient clinics or even pharmacies for the management of chronic autoinflammatory diseases: chronic diseases are considered as the greatest threat to human health, and include many common conditions such as diabetes, cardiovascular diseases, cancers, arthritis and even allergic reactions.[16] Having a biosensor readily available that can tell patients and professionals when an inflammatory reaction is worsening would not only help to alleviate emergency room overcrowding but it would also help professionals triage urgent cases since it will be able to tell at which inflammatory stage the patient is. A more detailed and validated value proposition can be found in Appendix 11.

Business feasibility

Initially, BiosensUM's start-up will be divided in three units, each mentored by our affiliated universities: business (mentored by the HEC Montréal), science (mentored by the UdeM), and technology (mentored by the Polytechnique Montréal). The Business unit will be divided in two departments: "Marketing and Public Relations", which will take care of the marketing strategies (social media presence), funding and communication with stakeholders' representatives; and the "Legal & Patent" department will take care of patent filing and building an IP strategy in accordance with local and international regulations. The Science and Technology units will be working on R&D and on the optimization of initial prototype. The Scientific unit can count on the expertise of UdeM's Chemistry Dept.'s experts as well as counselling from Affinité Instruments' experts. The Technology unit is also interested in extending their business relations with our main suppliers such as Ocean Optics for the detector(s) and Thor Labs for optical supplies (fibers, connectors, etc.): these partnerships would benefit both sides as it would allow BiosensUM to save more funding that could be redirected to other needs and it would allow these tech companies to take a stand in the forefront of the biomedical scene.

Based on the CIPO's five-year business strategy and financial projection, the first two years will be dedicated to R&D, patent filing, IP strategy and building a solid marketing strategy outside of local implementation. During those initial years, the Legal & Patent dept. will be working closely with the MSSS to implement our innovative technology in the healthcare network. Based on our initial discussions with our local (Quebec's) Department of

Biovigilance and Medical Biology, it is possible for our project to apply for government funding[37] through the FSISSS[1] offered by the Bureau de l'Innovation (BI) and build a professional network through government-funded events such as the Grand Rendez-vous de l'innovation Québécoise. Considering the current limitations of our local market (such as the classification of IL-6 quantification as "superregional"), the Legal dept. will most importantly need to register BiosensUM under Quebec's lobbyist registry in order to submit our new POCT for IL-6 quantification to the BI: since our proposal has the potential to change and standardize current procedures within our local government, it will also be an opportunity to open new doors for innovative projects in the health industry. After building the legal foundations of our project, the Business unit will be able to work more consistently in the future with our university's R&D incubator (BRDV) as well as with the governments' BI. Thereafter, the Marketing & Public Relations dept. will plan on an efficient commercialization strategy while targeting different customer segments: as mentioned previously, since the main adopters are hospital-setting prescribers (clinical nurses and doctors), we plan on starting with a B2B2C marketing strategy to limit the financial step-backs of direct sales.

This B2B2C consists of using another business, such as an R&D incubator or another key partner, as a channel to reach our main adopters: since most protocols are based off local regulations and governmental recommendations, our collaboration with the MSSS and BI will not only give us more credibility but also more opportunities to share our POCT with healthcare institutions locally. Since HAs also take into consideration their professionals' recommendations for new equipment and protocols, BiosensUM counts on various networking events during the first two years to share its innovations with experts from various horizons: one of these events will notably take place in September, as part of the Forces Avenir grant, which BiosensUM has a chance to win up to 20,000$ CAD.

Financial viability

As for the financial viability of our project, as mentioned earlier, current alternatives on the market are based on blood testing, which can cost anywhere between 150 to 3,000$ CAD: these prices vary depending on the health insurance benefits as well as the test conducted and if it is conducted in a private or public setting. When comparing regular blood tests to less commonly used IL-6 quantification (in clinical settings), it is possible to see a huge difference in the cost of a single test: depending on the Canadian laboratory that conducts the experimentation (public or private), IL-6 quantification costs from 19,72$ to 47,70$, which is at least 1/3 of the cost of blood testing.[38] Knowing that sepsis costs Canadians up to 1,7B$ CAD annually[39,40] (long-term ICU stays, management of multiple comorbidities, etc.) allows us to estimate the cost per hospital since there are about 1,300 hospitals in Canada: which brings sepsis-related costs to about 1,3M$ CAD per hospital per year. According to machine learning predictions, early sepsis detection can save up to 75 lives for an individual ICU with 50 beds and reduce sepsis-related costs by 560k$ US, which amounts to about 715k$ CAD, reducing sepsis-related costs by almost half.[41]

Our proposed biosensor would considerably help alleviate the financial burden of sepsis: although we offer a prototype that will still need optimization (more specifically in the optical system, which we planned on building ourselves) to reduce its labor cost of 1,200$ CAD (and reseller price at 2,, our new lab-on-a-tip technology allows us to carry out IL-6 quantification tests for less than 5$ CAD, allowing for an economy of more than 10$ CAD per test compared to current alternatives on the market. Based on our five-year financial projection built with the help of a business accountant from Bowker Capital Inc. (see Appendix 14), our first two years are, as expected, in financial deficit. However, starting our 3rd year, we are planning to reach up to 20% of the Canadian market (75,000 annual sepsis cases) by selling 260 biosensors and 45,000 cartridges (lab-on-a-tip). From then on, we plan on growing outside of the Canadian market, start making net profit and growing to 25% by 2026 and 30% by 2027. Although these numbers may seem like a lot, it is because of the great need of a fast solution for IL-6 quantification in the current POCT market as well as the lack of direct competition since there is no other testing offered for the diagnosis of sepsis, as mentioned earlier. Although the sepsis diagnostic market is already offering a lot, BiosensUM also plans on opening up to other markets in the first years of sales. In fact, the implementation of our biosensor would not only help alleviate the financial burden of sepsis but also that of many diseases in which inflammation is involved: from the risk assessment of traumatic injuries to the monitoring of chronic inflammatory diseases (cancers, arthritis, diabetes, etc.), our biosensor has the potential to also alleviate the financial burden associated with chronic illnesses (see Appendix 15).

[1] Fonds de soutien à l'innovation en santé et en services sociaux

Apart from the healthcare industry, IL-6 has also been explored in the sports industry, as the cytokine is also released during exercise and can help predict muscle recovery and athletic performance, which makes it an additional market in which our biosensor can thrive into.[42,43]

Our biosensor has not only the possibility to introduce an innovative and rapid point-of-care technology for the quantification of IL-6 that has never been marketed before, but, from a broader perspective, it can also bring standardization in the diagnosis and management of acute inflammatory reactions such as sepsis since its time-to-result surpasses all current alternative tests, by accurately quantifying IL-6 in 5 minutes.

3 - Team and support

Contribution of the Team Members

- Pierre-Alexandre Aubé is the team co-captain and a member of the technological subunit and has worked on the optics designs, designs of the 3D parts impression, the coding, and the integration of our biosensor.
- Maryam Bani-Otero was present to some meetings and team bonding activities.
- Ryma Boudries is the team co-captain and a member of the scientific subunit and has been one of the main members to work in the lab to realize our assays, she provided protocols for the nanoparticle's synthesis, the coating and antibody conjugation. She is also a member of the entrepreneurial subunit and has helped a lot to the completion of our entrepreneurial goals and deadlines.
- Katia Cherifi is the head of the entrepreneurial subunit and by far main actor of it. She is also a member of the scientific subunit and is the team promotion person who led our presence on social media.
- Jade Cimmino is part of the technological subunit who worked on the assembly of our biosensor and helped with the team promotion by filming and editing our 1-minute pitch.
- Emmanuelle Del Guidice was present to some meetings and team bonding activities.
- Caroline Dubois is part of the scientific subunit, she helped with the nanoparticle's synthesis, the coating and antibody conjugation optimization. She also contributed considerably to the thinking process for the prototype.
- Sarrah Ferragne is part of the business subunit, she also helped with the deadline management.
- Faten Idar is part of the technological subunit, she made all the models and the 3D prints and helped with the redaction of the code to plot the results.
- Christopher Ledo was part of the scientific subunit, he helped with the nanoparticle's synthesis and SPR measurements.
- Marwa Safa was part of the scientific subunit, she helped with the nanoparticle's synthesis and SPR measurements.
- Jessy Wan was present to some meetings and team bonding activities

People who have given their support

· Jean-François Masson (team supervisor) introduced the currently available biosensor concepts on the market. He was readily available to answer our questions and evaluate our suggested prototype ideas.

· Alexandrine Frappier (team mentor) was present to form some team members and was very supportive throughout the whole competition. Her knowledge that came from being last year's team captain was very useful.

- Claude Frappier, a business accountant from Bowker Capital Inc. gave us information needed to complete the financial plan and helped create our 5-year projection.

- Pierre Chaurand, a professor in the Department of Chemistry at the Université de Montréal with expertise in methodologies development for the analysis of the molecular content of thin tissue sections by mass spectrometry, who helped us by proofreading the TRD.

- Frédéric Fournelle, a member of BiosensUM 2019 and 2020, who helped us by proofreading the TRD.

- Maryam Hojjat Jodaylami, PhD student in Lab Masson who helped us acquire SEM images.

Sponsors and partners

· Université de Montréal (incl. Polytechnique Montréal, Collectif Social, ASEQ, FAECUM and all departments we have contacted): It is safe to say that without the generous help of our universities, both through funding and having access to various labs and equipment, this project would not have been possible.

· Hytest (SensUs partner) provided us proteins and antibodies

·The SPR & Plasmonic Biosensors research group (Pr. Jean-François Masson): http://www.sprbiosensors.com/ provided a lot of information on the technologies explored, materials, equipment and lab space for biological and chemical assays.

4 - Final Remarks

We would like to wholeheartedly thank our supervisor Jean-François Masson and our coach Alexandrine Frappier for their advice and guidance throughout the project; our partners for providing us with good options and solutions to the many aspects involved in the conception of our biosensor; SensUs organization for having a very interesting and impactful project that allowed us to expand our knowledge

Although COVID-19 came with its fair share of restrictions such as having a limited number of people in the labs or having to do most of our meetings online, we got to collaborate wonderfully, and have a memorable experience. This project has inspired a few of us to want to further explore the domain of biosensors in our future endeavors. After the competition, some team members would be really interested in further developing our biosensor.

In the technological aspect of our biosensor, we would like to switch our LCD display for an LED display, which would be safer both for our users as LED displays have smaller backlighting and are much safer for the eyes, and for the environment as LCD displays require mercury for their production [44].

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Appendix

Appendix 1: Protocol for the conception of the TBF.

The nanofibers were obtained by pulling borosilicate glass rods with a P-2000 laser electrode stretcher (Sutter Instrument, California) using a two-line program with different parameters (specific to the instrument). For peak diameters of about 200 nm:

Line 1 : Heat = 280, Filament = 0, Velocity = 15, Delay = 200, Pull = 20.
Line 2 : Heat = 350, Filament = 0, Velocity = 15, Delay = 128, Pull =150.

The nanofibers were first immersed in acetone for 10 minutes, then in ethanol for 10 minutes. Subsequently, they are immersed in a solution of freshly prepared piranha, a mixture 3: 1 v / v of sulfuric acid (H_2SO_4) and hydrogen peroxide 30% (H_2O_2) at 90 ° C for 90 minutes. WARNING: the piranha solution is very corrosive and must be handled with care. Then rinsed thoroughly with Milli-Q water and dried for 60 minutes.

A solution of 0.05 mg/mL of the di-block PS-P4VP in tetrahydrofuran (THF) was prepared from a stock solution of 1 mg/mL in THF. The clean, hydrophilic nanofibers were immersed in the polymer solution using a dip-coater (KSV3000 Langmuir-Blodgett) at a speed of 51.4 mm/minute. After an immersion of 3.5 minutes, the polymer-coated nanofibers were removed from the polymer solution at the same speed and placed in a petri dish. The polymer-coated nanofibers can then be stored in the dark at 4°C until they are used.

Then, the metallic nanoparticles are functionalized on the fibers. The AuNPs were first concentrated and then redispersed in a 3 mM solution of sodium citrate before adjusting the pH of the nanoparticle solution to about 4.5 using HCl. The fibers are then immersed in the nanoparticle solutions for 2 hours. After two hours, the nanoparticle-coated nanofibers were rinsed with Milli-Q water to remove the unbound nanoparticles and air-dried. (Photos of the fibers were taken using a JEOL JSM-7400F field emission scanning electron microscope (SEM).)

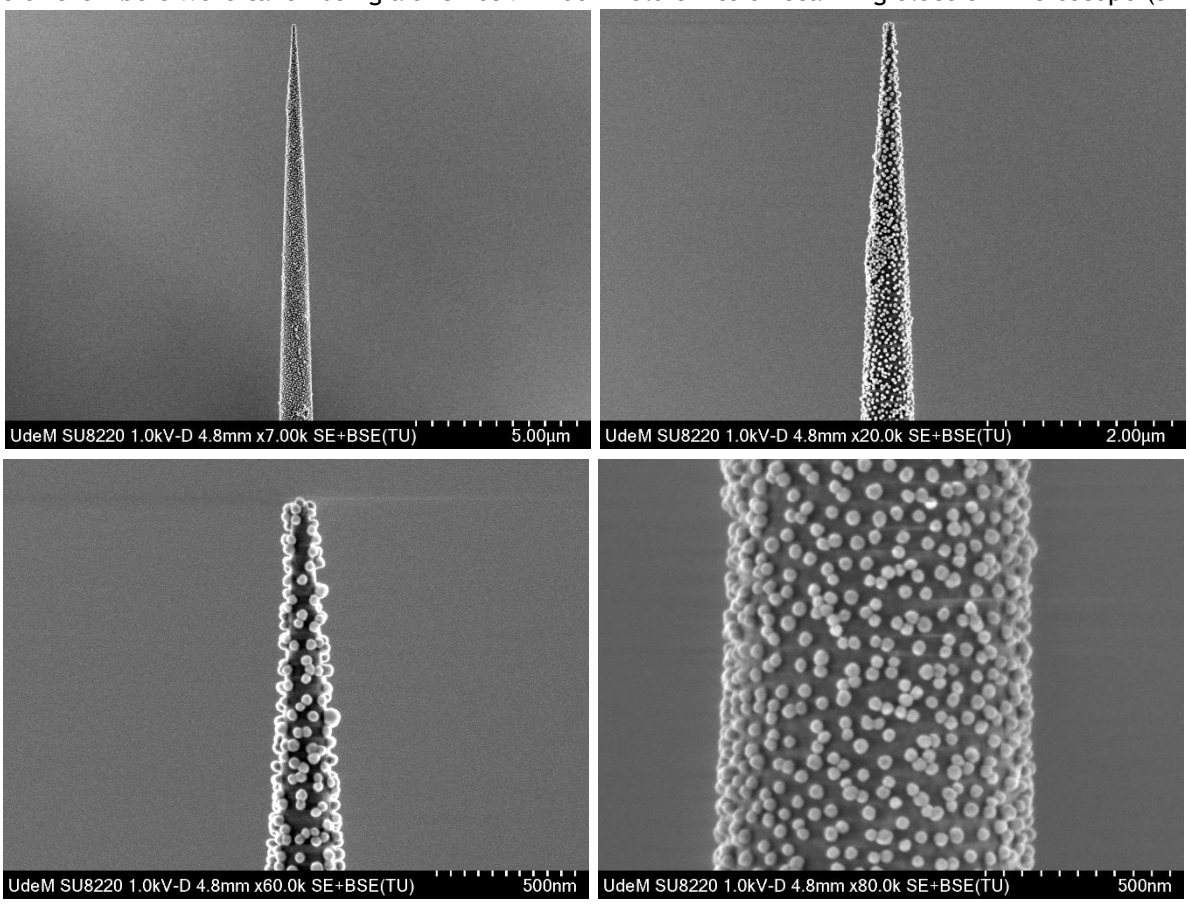


Figure 6 – Images of the 40 nm AuNPs coated TBF acquired using a JEOL JSM-7400F field emission scanning electron microscope.

Appendix 2: UV-VIS measurements for AuNPs size accuracy.

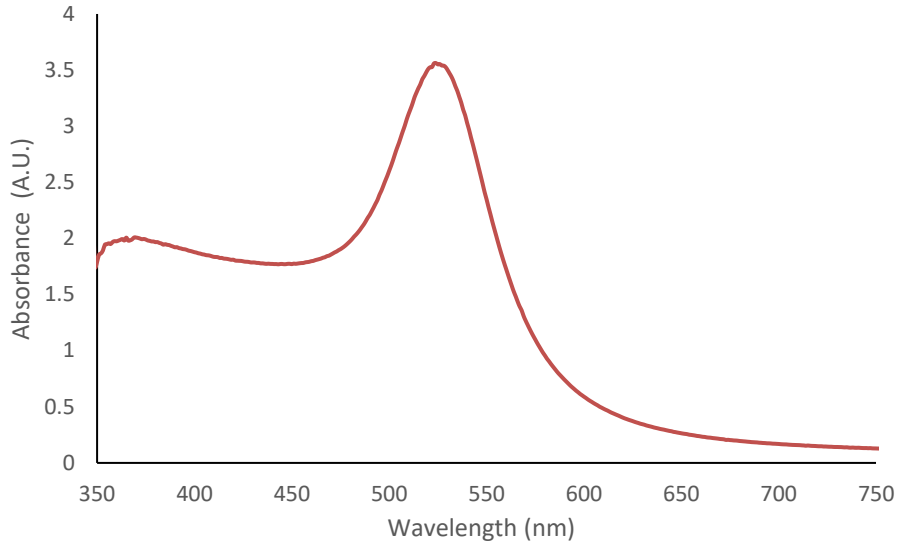
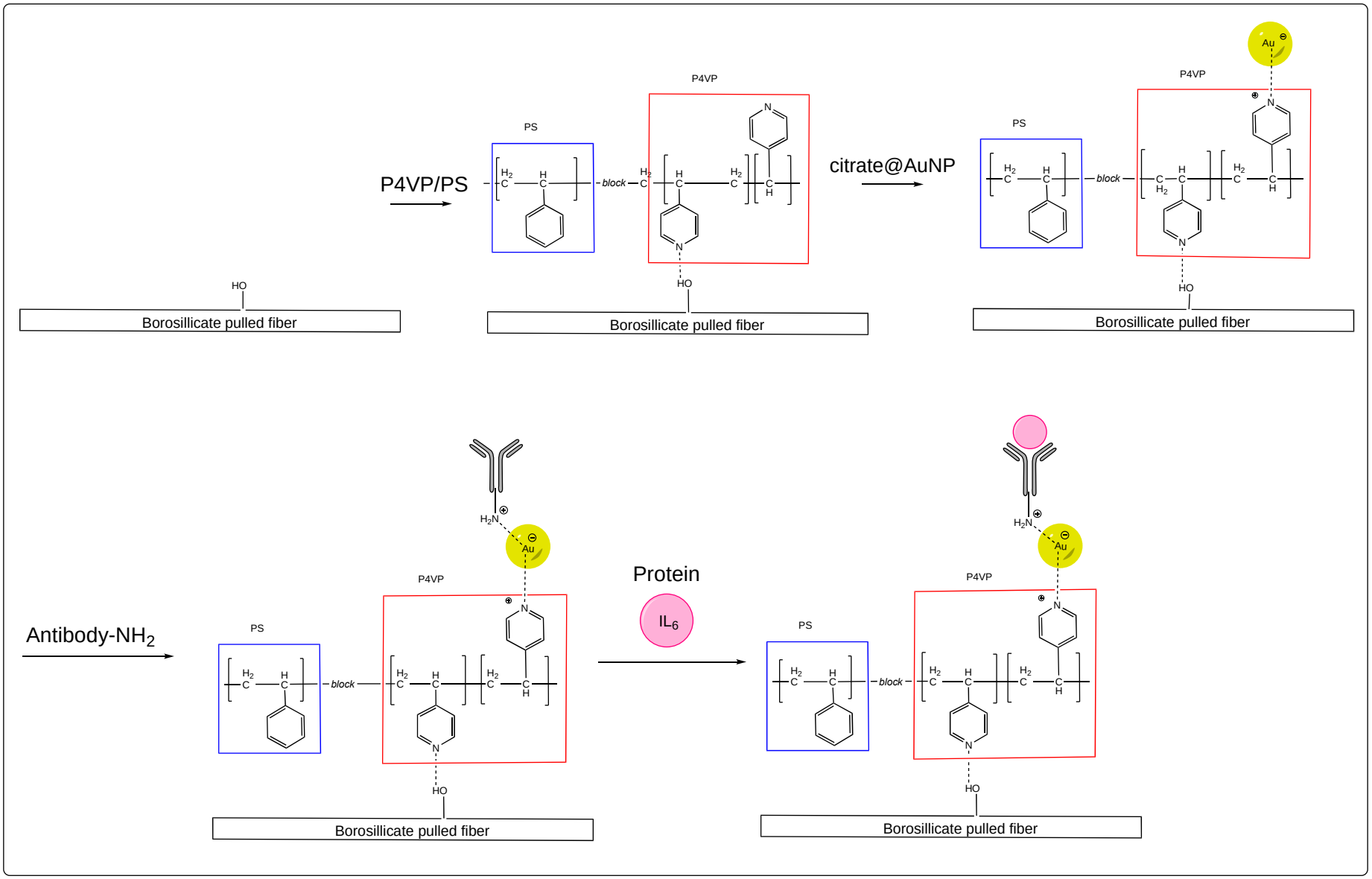


Figure 7 - Absorbance spectrum of the spherical gold nanoparticles after 6 additions (529.2 nm) acquired with a UV-vis-NIR Agilent Technologies Cary 6000i.

Appendix 3: Reaction dynamics to coat the TBF.



Appendix 4: Concept design of an improved microfluidic cartridge

Below is a concept design for a microfluidic cartridge that contains our TBF. This would greatly increase the ease of use of our device as the tip could be enclosed but still interact with the sample. Unfortunately, we did not have enough time to implement it, but we are still confident that this design is the best option for the future of our technology.

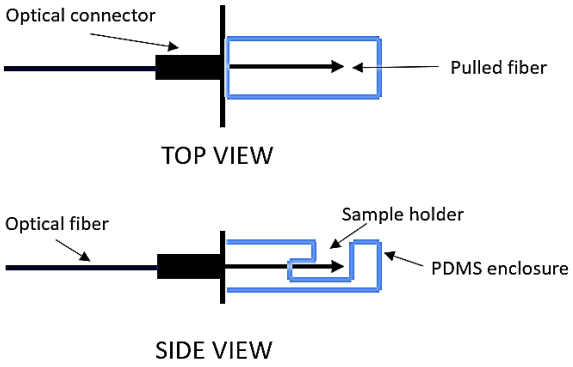
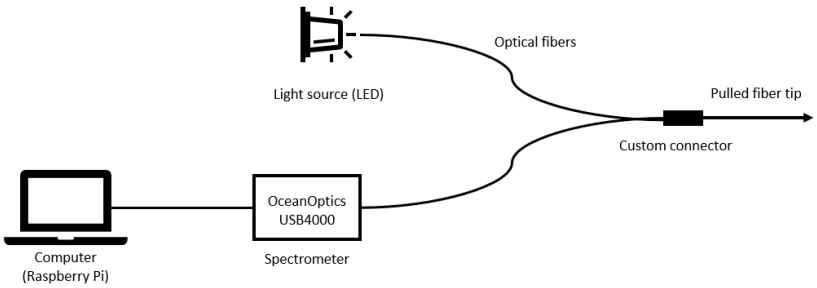


Figure 9. Scheme of a future microfluidic cartridge design.

Appendix 5: Optical setup

Below is a figure showing the different parts that compose our optical setup. It contains a light source, LEDs in our case, a custom-built connector to connect with our pulled fiber tips and a spectrometer to digitize the signal that connects directly in a computer.

Schematic representation of our optical setup:



Study on the impact of light intensity:

In LSPR, the light intensity can have a huge impact on the signal quality which is why we decided to evaluate the impact light intensity on the signal captured. We used a potentiometer to control our LEDs and chose 4 levels of intensity and measured the output spectrum at those levels. The resulting curve is shown below. As we can see, the noise doesn't increase a lot which is why we chose the highest intensity since the advantage of lowering the intensity is to reduce the noise.

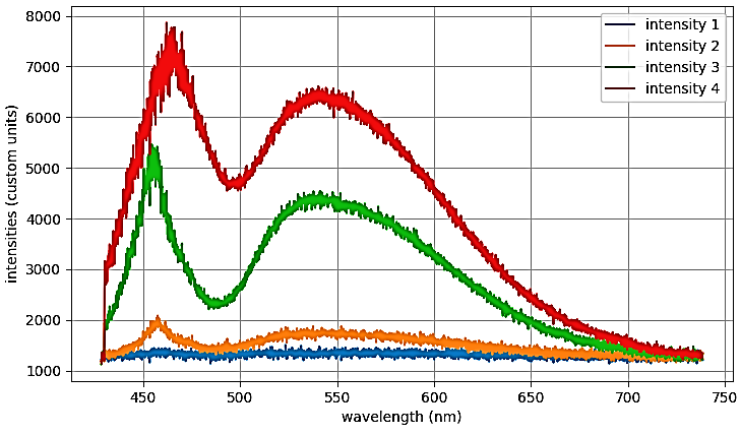


Figure 8 – Light intensities spectra acquired with an Ocean Optics USB4000 UV-Vis.

Appendix 6 : Calibration values.

Table 2. Calibration values obtained with our biosensor.

[IL-6] (pg/mL)	λ_1 (nm)	λ_2 (nm)	λ_3 (nm)	Mean (nm)	Standard Deviation (nm)	Confidence Interval (nm)	Compliance ratio [1]
5	N/D [2]						
55	500.0	497.0	496.3	4978	2.0	5.0	84.4
150	497.8	498.4	497.2	497.8	0.6	1.0	293.6
600	497.4	498.5	498.9	498.3	0.8	2.0	206.7
1000	499.6	499.7	499.1	499.4	0.3	0.7	568.2
1500	502.0	501.8	502.2	502.0	0.2	0.5	917.9

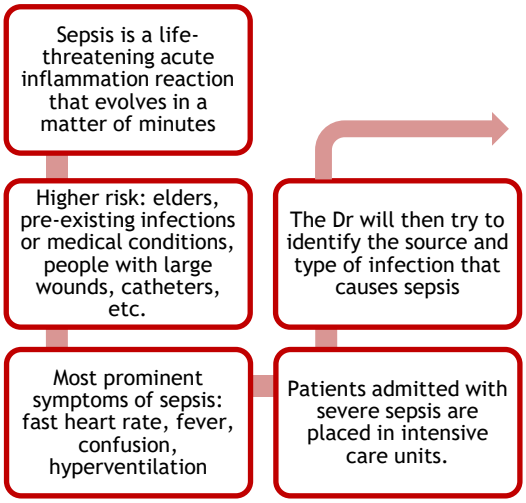
1. A compliance ratio ("Ratio de conformité") > 10 indicates that the actual detection limit of the method is lower than the detection limit estimated during the tests.
2. The tests were inconclusive.

Appendix 7 : Python code that drives the biosensor.

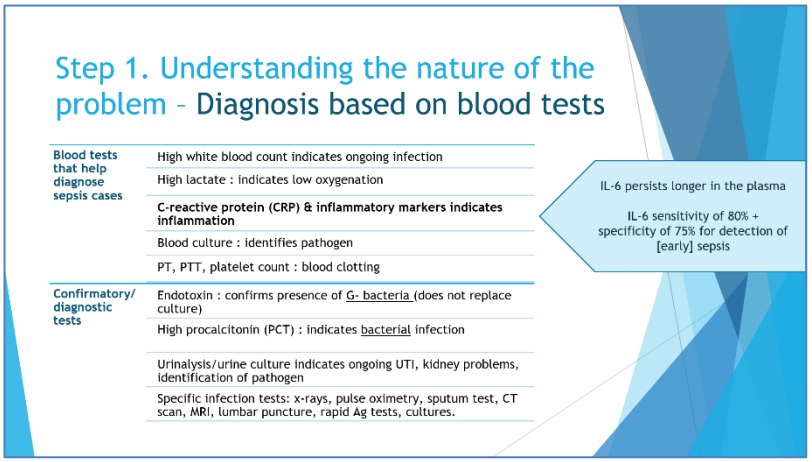
The code behind all our biosensor was developed in Python which we chose because it is both easy to use and well documented. We were able to find numerous libraries to help with different tasks like SciPy which we use for the digital filtering and Seabreeze which we use for the interaction with the OceanOptics USB4000. On top of the code written for the biosensor, a lot of code was written to perform various experiments with our setup. Since we highly believe in the value of open-source science, our team has decided to host all our code on GitHub and make it available to all. We hope that this contribution will help other teams of this competition in the future or fuel other scientific endeavours. Shown below is a list of the main functions used to perform our tests, but all the code can be found at https://github.com/paaube/biosensum_2022.

```
import matplotlib.pyplot as plt
import pandas as pd
import numpy as np
from scipy.signal import savgol_filter

from seabreeze.spectrometers import Spectrometer

def get_spectre():
    spec = Spectrometer.from_first_available()
    spec.integration_time_micros(20000)
    x = spec.wavelengths()
    y = spec.intensities()
    plot_spectre(x, y)
    output = pd.DataFrame(index=["wavelengths", "intensities"], data=[x, y])
    return output
def plot_spectre(x, y):
    fig, ax = plt.subplots()
    ax.plot(x, y)
    ax.set(xlabel='wavelength', ylabel='intensities', title='OceanOptics light spectrum')
    ax.grid()
    plt.show()
def save_to_pkl(df, filename):
    df.to_pickle(f"data/{filename}.pkl")
def load_pkl(filename):
    return pd.read_pickle(f"data/{filename}.pkl")
def absorbance(ref, test):
  ref = load_pkl(ref).T
  test = load_pkl(test).T
  test = test.assign(absorbance=filter_data(np.ravel(np.log10(ref[['intensities']] / test[['intensities']])))).drop(
      columns=['intensities']
  )
  test.plot(x='wavelengths', y='absorbance')
  plt.show()
  return test
def filter_data(df):
  return savgol_filter(df, 105, 3)
def plot_spectre_4(x1, y1, x2, y2):
    fig, ax = plt.subplots()
    ax.plot(x1, y1)
    ax.plot(x2, y2)
    ax.set(xlabel='wavelength', ylabel='intensities', title='OceanOptics light spectrum')
    ax.grid()
    plt.show()

def plot_spectre_2(x1, y1):
    fig, ax = plt.subplots()
    ax.plot(x1, y1)
    ax.set(xlabel='wavelength (nm)', ylabel='intensities (custom units)', title='Filtered light spectrum')
    ax.grid()
    plt.show()
```

Appendix 8. Customer journey



Alternatives on the market	Time-to-result	Price (per unit or per test)
Blood tests (inflammatory markers)	1-24 hours	150-3,000$ CAD
IL-6 Quantification (RIA -Govnt. Labs)	24 hours	20-50$ CAD
Tests usually required for sepsis cases	**Price**	
Blood tests	150-3,000$ CAD	
Urine tests	120$ CAD (on average)	
X-rays	366$ CAD (on average)	
CT scans	375-775$ CAD (not covered by public insurance)	
IV fluids	300$ CAD (on average)	



Schema. The customer journey begins when a patient is admitted with [severe] sepsis: depending on the symptoms, medication may be started right away. However, because of the risks associated with sepsis, routine testing is required at the very least: blood test (culture) or urine testing in order to identify the cause of sepsis (most likely infection). If the patient has a bacterial infection, antibiotic therapy will be initiated. In most severe sepsis cases, additional procedures such as IV therapy or even scans may be required, which will bring the end price way higher for the patient (especially in cases which health insurance does not cover some services) than our alternative.

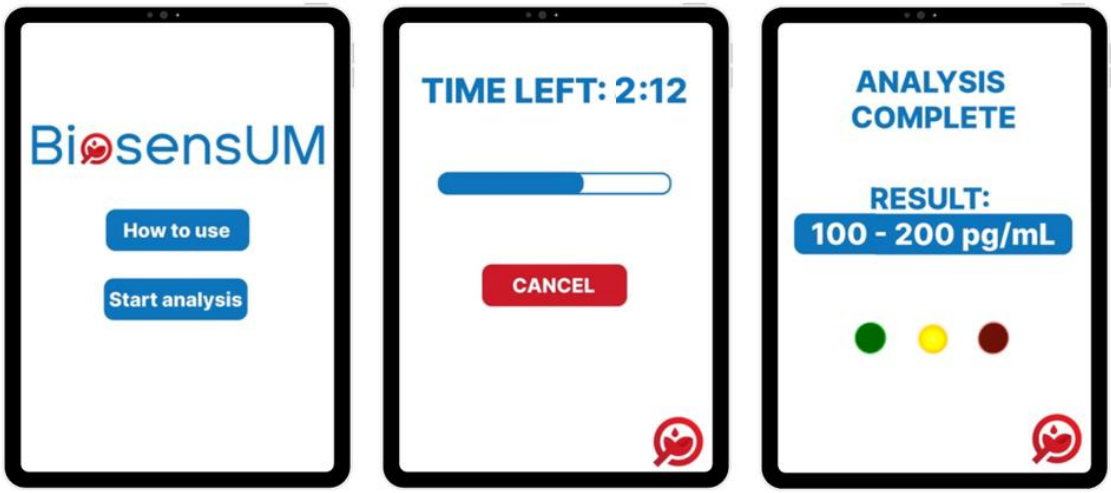
Appendix 9. Cost calculations of the Cartridge and Device

	Material for lab-on-a-tip (1)	Price per unit	Price per cartridge (lab-on-a-tip)
Cartridge	Borosilicate fibers	45$ per 250 fibers = 500 tips	0,09$
	Gold nanoparticles	593$ per 5 grams	4,48$
	Coating polymer	500$ per 1 gram	0,13$
	Monoclonal antibody anti-IL-6	896$ per 400 µL	0,02$
	TOTAL COST PER TIP (PER TEST)		**4,71$**
	Material for prototype (biosensor)	**Price per unit**	**Price per prototype**
Device	Ocean Optics	1,000$ per unit	1,000$
	Optical fibers	2,59$ per meter	3,89$
	Epoxy	5$ per 2 grams	0,60$
	PLA	40$ per 1kg	40$
	Raspberry Pi 4	75$ per unit	75$
	Touchscreen	80$ per unit	80$
	TOTAL COST PER DEVICE		**1,199.49$**

Details of the raw production cost (material)

Appendix 10. Device interface

User interface

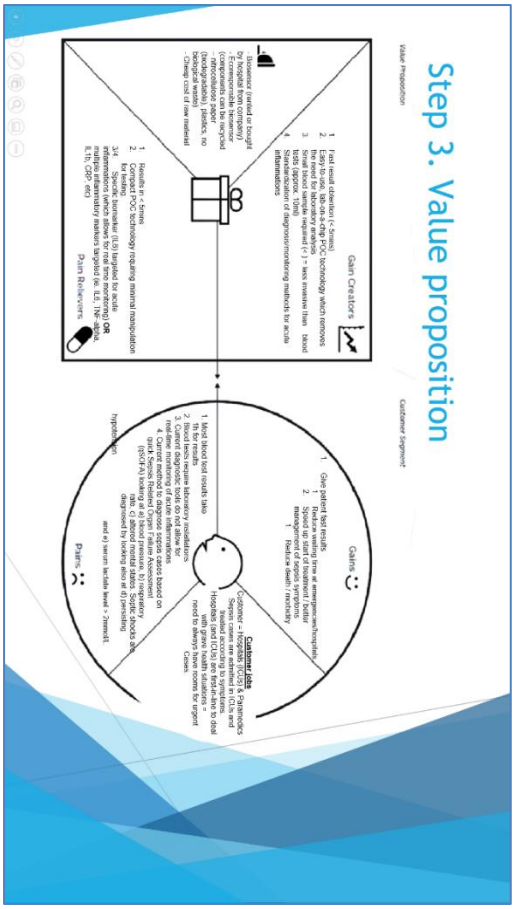


Clinical values of IL-6 (reference values based on literature - to be optimized with more research)

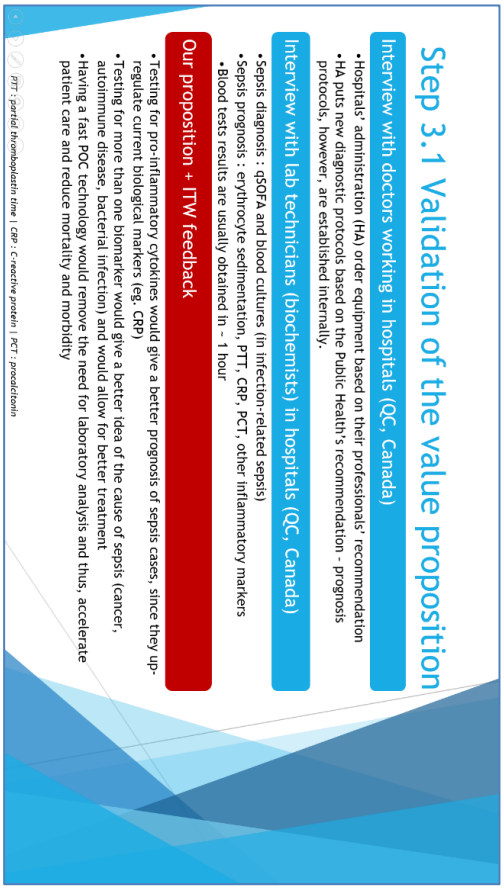
Colour code	Sepsis stage	Value range	Possible complications
Green	Normal levels	0 - 52,6 pg/mL[1,38]	N/A
Yellow	Early to moderate sepsis	52,7 - 348,8 pg/mL	Risk of organ damage
Red	Severe sepsis	> 348,9 pg/mL	Risk of organ failure / death

Appendix 11. Value proposition

Initial value proposition map



Sum-up of interviews with the main adopters



Appendix 11. Value proposition

Proposed biosensor

- Innovative lab-on-a-tip technology that has never been commercialized before, which makes it an interesting POCT
- Easy-to-use POCT that doesn't require specific expertise to operate
- Competitive time-to-result with IL-6 quantification in 5 minutes
- Inexpensive solution (production: 5$ CAD per test | 1,200$ CAD per device) to laboratory analysis (150-3,000$ CAD)
- BiosensUM wishes to develop a microfluidic system that would allow us to do triplicates on the same sample for additionnal precision
- We are also looking into multiplexing to test for multiple biomarkers to maximize the information obtained from a sample but also to run multiple samples at once for time-efficiency

Appendix 12. Financing in Canada and Quebec

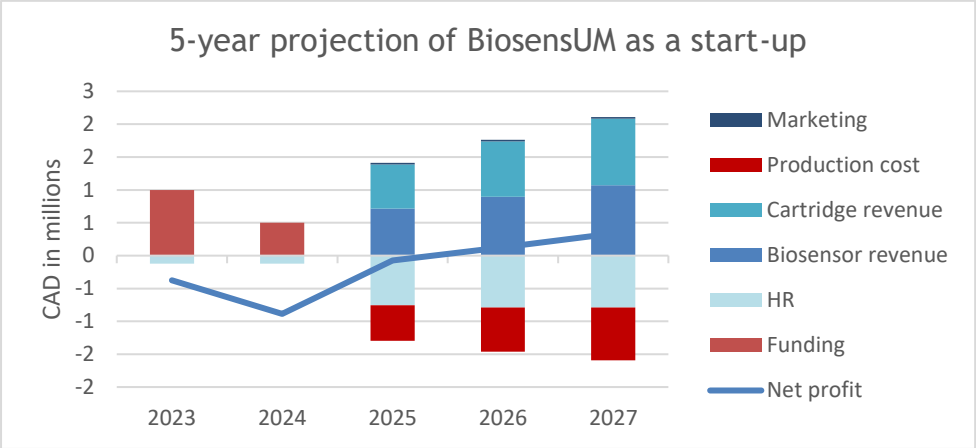
Here are grants and loan opportunities in Canada or Quebec for our start-up:

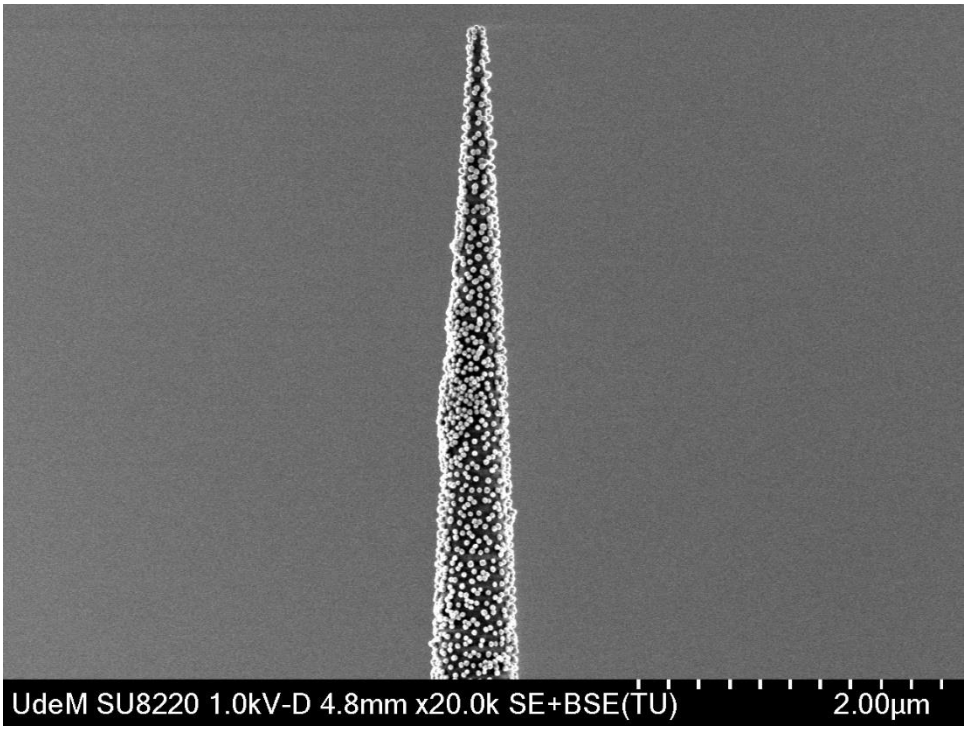
- SADC and CAE (youth strategy fund): organization offering up to 50 000$ to entrepreneurs between 18-39 years old
- Industrial Research Assistance Program (IRAP): federal program giving a grant up to 60%-80% of internal technical labour and subcontractor expenses.
- Bureau de l'Innovation (Government of Quebec): up to 700 000$
- Bank for Canadian entrepreneurs (BDC): loan up to 100 000$.
- Idea to Innovation Grants: federal program funding up to 50% of R&D.
- PME MTL-Young Business: offers up to 15 000$ to help start-up.
- Investissement Québec: up to 50 000$ to help small businesses.
- Canada Small Business Financing Program: up to 350 000$ in loans with 3% interest.

Appendix 13. Health Canada regulations

Health Canada regulates every medical product available on the Canadian market. This means our biosensor must be reviewed by this organization to sell it in Canada. Our biosensor belongs to the Class III medical device for a near patient in vitro diagnostic which costs 16 032$ for a license then we must pay a fee of 381$ per year to keep the license active. This investigation takes about 60 calendar days before reaching a first decision which could be either requesting additional information, approving the application or refuse the application.[45]

Appendix 14. Five-years financial projections


5-year projection of BiosensUM as a start-up
CAD in millions
3
2
2
1
1
0
-1
-1
-2
-2
2023
2024
2025
2026
2027
Marketing
Production cost
Cartridge revenue
Biosensor revenue
HR
Funding
Net profit



Appendix 13. Five-years financial projections

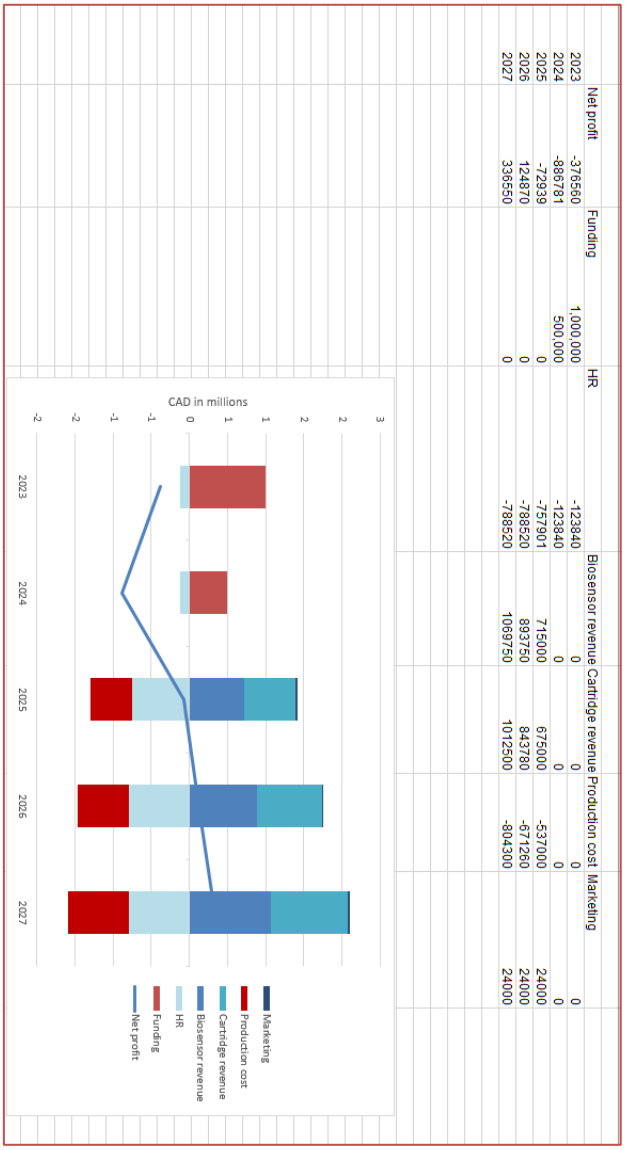
Details of the end result of the 5 years overview of costs and revenues

DO NOT CHANGE FIELDS THAT ARE NOT YELLOW		Basis for Assumptions	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11	Month 12	Quarter5	Quarter6	Quarter7
Human Resources		Base Unit Cost per month unit = 1 full-time Salary or fee	enter # of HR units/month, not £												enter # of HR units/month, not £		
1 *Chemist/biochemist senior*		5,600	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	3.0	3.0	3.0
3 *Biomedical engineer*		5,280	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	3.0	3.0	3.0
4 *Engineer - Software and data*		5,280	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	3.0	3.0	3.0
6 *Business manager*		4,480	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	3.0	3.0	3.0
Number of FTEs (Full-Time Equivalents)			4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	12.0	12.0	12.0
Revenue hypothesis	**Unit Price of Product or service**	**Type of Unit**	**Enter Revenue per month (in units of revenue, not £)**												**Enter Revenue per quarter (in units of revenue, not £)**		
Enter Type of Product or service																	
A *Biosensor*	2,750	*product*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B *Cartridges*	15	*product*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total revenues			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			Cost per month												**Cost per quarter**		
DIRECT VARIABLE COSTS	**Direct cost per unit of product**																
A *Biosensor*	1,200		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B *Cartridges*	5		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C *0*	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D *0*	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E *0*	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total direct costs			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GROSS PROFIT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FIXED COSTS																	
A **Human Resources**																	
Fixed remunerations			20,640	20,640	20,640	20,640	20,640	20,640	20,640	20,640	20,640	20,640	20,640	20,640	185,760	185,760	185,760
Index and accumulated salary increase	2.00%		0	0	0	0	0	0	0	0	0	0	0	0	3,715	3,715	3,715
Bonus	0.00%	of turnover	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Subtotal			*20,640*	*20,640*	*20,640*	*20,640*	*20,640*	*20,640*	*20,640*	*20,640*	*20,640*	*20,640*	*20,640*	*20,640*	*189,475*	*189,475*	*189,475*
Total Human Resources			20,640	20,640	20,640	20,640	20,640	20,640	20,640	20,640	20,640	20,640	20,640	20,640	189,475	189,475	189,475
B **Training expenses**																	
Training expenses			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Training expenses			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C **Services rendered by third parties**																	
FDA certification (40)	40	per month	40	40	40	40	40	40	40	40	40	40	40	40	120	120	120
Accountant (4200)	4,200	per month	4,200	4,200	4,200	4,200	4,200	4,200	4,200	4,200	4,200	4,200	4,200	4,200	12,600	12,600	12,600
	0	per month	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	per month	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Services rendered by third parties			4,240	4,240	4,240	4,240	4,240	4,240	4,240	4,240	4,240	4,240	4,240	4,240	12,720	12,720	12,720
D **Infrastructure and operational costs**																	
Manufacturing overheads (5%)	0	per month	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ICT (500)	500	per month	500	500	500	500	500	500	500	500	500	500	500	500	1,500	1,500	1,500
Office/rent (2000)	2,000	per month	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	6,000	6,000	6,000
Insurance (4000)	4,000	per month	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000	12,000	12,000	12,000
	0	per month	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	per month	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	per month	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	per month	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Infrastructure and operational costs			6,500	6,500	6,500	6,500	6,500	6,500	6,500	6,500	6,500	6,500	6,500	6,500	19,500	19,500	19,500
E **Marketing**																	
All marketing (8300)	2,000	per month	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
e.g. Webdesign / Logo / Corporate Identity	0	per month	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
e.g. Recruitment budget	0	per month	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
e.g. Fairs	0	per month	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
e.g. Internet Marketing / Open Source	0	per month	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
e.g. General Marketing & Travel Budget	0	per month	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

DO NOT CHANGE FIELDS THAT ARE NOT YELLOW	Basis for Assumptions	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11	Month 12	Quarter5	Quarter6	Quarter7
Total Marketing		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SUMMARY																
Total revenues		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total direct costs		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total gross margin		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Human Resources		20,640	20,640	20,640	20,640	20,640	20,640	20,640	20,640	20,640	20,640	20,640	20,640	189,475	189,475	189,475
Total Training expenses		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Services rendered by third parties		4,240	4,240	4,240	4,240	4,240	4,240	4,240	4,240	4,240	4,240	4,240	4,240	12,720	12,720	12,720
Total Infrastructure and operational costs		6,500	6,500	6,500	6,500	6,500	6,500	6,500	6,500	6,500	6,500	6,500	6,500	19,500	19,500	19,500
Total Marketing		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total costs (excluding depreciations)		31,380	31,380	31,380	31,380	31,380	31,380	31,380	31,380	31,380	31,380	31,380	31,380	221,695	221,695	221,695
Operating Profit excl. depreciations		-31,380	-31,380	-31,380	-31,380	-31,380	-31,380	-31,380	-31,380	-31,380	-31,380	-31,380	-31,380	-221,695	-221,695	-221,695

DO NOT CHANGE FIELDS THAT ARE NOT YELLOW	Quarter8	Quarter9	Quarter10	Quarter11	Quarter12
Human Resources					
1 *Chemist/biochemist senior*	3.0	3.0	3.0	3.0	3.0
3 *Biomedical engineer*	3.0	3.0	3.0	3.0	3.0
4 *Engineer - Software and data*	3.0	3.0	3.0	3.0	3.0
6 *Business manager*	3.0	3.0	3.0	3.0	3.0
Number of FTEs (Full-Time Equivalents)	12.0	12.0	12.0	12.0	12.0
Revenue hypothesis					
Enter Type of Product or service					
A *Biosensor*	0	65	65	65	65
B *Cartridges*	0	11250	11250	11250	11250
C	0	0	0	0	0
D	0	0	0	0	0
E	0	0	0	0	0
Total revenues	0	347,500	347,500	347,500	347,500
DIRECT VARIABLE COSTS					
A *Biosensor*	0	78,000	78,000	78,000	78,000
B *Cartridges*	0	56,250	56,250	56,250	56,250
C *0*	0	0	0	0	0
D *0*	0	0	0	0	0
E *0*	0	0	0	0	0
Total direct costs	0	134,250	134,250	134,250	134,250
GROSS PROFIT	0	213,250	213,250	213,250	213,250
FIXED COSTS					
A Human Resources					
Fixed remunerations	185,760	185,760	185,760	185,760	185,760
Index and accumulated salary increase	3,715	7,505	7,505	7,505	7,505
Bonus	0	0	0	0	0
Subtotal	*189,475*	*193,265*	*193,265*	*193,265*	*193,265*
Total Human Resources	189,475	193,265	193,265	193,265	193,265
B Training expenses					
Training expenses	0	0	0	0	0
Total Training expenses	0	0	0	0	0
C Services rendered by third parties					
FDA certification (40)	120	120	120	120	120
Accountant (4200)	12,600	12,600	12,600	12,600	12,600
	0	0	0	0	0
	0	0	0	0	0
Total Services rendered by third parties	12,720	12,720	12,720	12,720	12,720
D Infrastructure and operational costs					
Manufacturing overheads (5%)	0	0	0	0	0
ICT (500)	1,500	1,500	1,500	1,500	1,500
Office/rent (2000)	6,000	6,000	6,000	6,000	6,000
Insurance (4000)	12,000	12,000	12,000	12,000	12,000
	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
Total Infrastructure and operational costs	19,500	19,500	19,500	19,500	19,500
E Marketing					
All marketing (8300)	0	6,000	6,000	6,000	6,000
e.g. Webdesign / Logo / Corporate Identity	0	0	0	0	0
e.g. Recruitment budget	0	0	0	0	0
e.g. Fairs	0	0	0	0	0
e.g. Internet Marketing / Open Source	0	0	0	0	0
e.g. General Marketing & Travel Budget	0	0	0	0	0

DO NOT CHANGE FIELDS THAT ARE NOT YELLOW	Quarter8	Quarter9	Quarter10	Quarter11	Quarter12
Total Marketing	0	6,000	6,000	6,000	6,000
SUMMARY					
Total revenues	0	347,500	347,500	347,500	347,500
Total direct costs	0	134,250	134,250	134,250	134,250
Total gross margin	0	213,250	213,250	213,250	213,250
Total Human Resources	189,475	193,265	193,265	193,265	193,265
Total Training expenses	0	0	0	0	0
Total Services rendered by third parties	12,720	12,720	12,720	12,720	12,720
Total Infrastructure and operational costs	19,500	19,500	19,500	19,500	19,500
Total Marketing	0	6,000	6,000	6,000	6,000
Total costs (excluding depreciations)	221,695	231,485	231,485	231,485	231,485
Operating Profit excl. depreciations	-221,695	-18,235	-18,235	-18,235	-18,235



2023	2024	2025	2026	2027
Net profit	-376560	-886781	-72939	124870	336550
Funding	1,000,000	500,000	0	0	0
HR	-123840	-123840	-757901	-788520	-788520
Biosensor revenue	0	0	715000	893750	1069750
Cartridge revenue	0	0	0	675000	843780	1012500
Production cost	0	0	0	-537000	-671260	-804300
Marketing	0	24000	24000	24000	24000

Appendix 14. Financial burden of chronic illnesses.[46]

Chronic illness	Approximate annual costs in the United States ($ USD)
Heart disease and stroke	216 B$
Cancer	240 B$
Diabetes	327 B$
Arthritis	140 B$
Alzheimer	305 B$