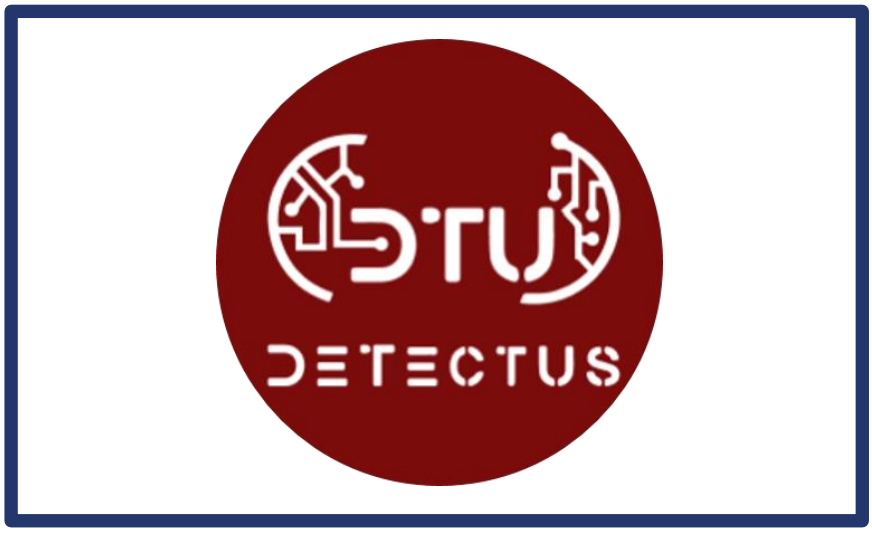
Team Results Document
DeTectUs



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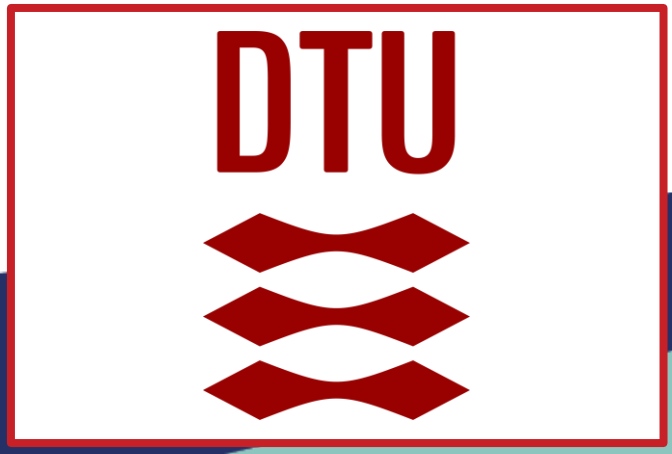
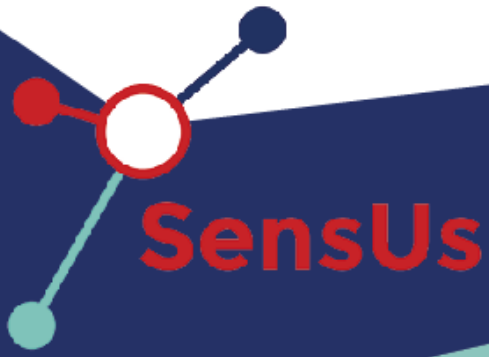
12.08.2022





Content

1. Abstract

Sepsis is a shapeshifting disease; it can take many forms and can affect anyone. According to the WHO (World Health Organization), 1 in every 5 deaths is related to sepsis [1]. Most of these deaths could have been prevented if there was an early, quick, and reliable method of detection of sepsis. In this context our team DeTectUs presents a novel biosensor technology for precise and fast measuring of IL-6 concentrations in blood plasma.

Our novel biosensor is a graphene field effect transistor (GFET) able to detect IL-6 concentrations going down to 1 pg/mL in a very short amount of time.

In this technology graphene is functionalized with pyrene tagged DNA-aptamer (PTDA) which permits only the measurement of the specific negative charges of IL-6 and not of the other ions in the human blood plasma. Once the IL-6 binds to the aptamers, the negative charges move nearer the graphene bridge, and a modulation of the graphene channel current occurs. The change in the current is influenced by the concentration of IL-6. The current is measured by a custom-designed reading instrument, with a further display of the results on Raspberry Pi 4.

The ultra-sensitivity of GFETs combined with the aptamers as the biorecognition sites can provide rapid and accurate information which could constitute an important milestone in the improvement of the sepsis diagnosis.

2. Biosensor system and assay

2.1 Physical Transduction

Conventional field effect transistors (FETs) have three main components namely the source, drain and gate. The source is a supplier of electrons and is connected to the drain by a channel. The drain acts as an electron sink and the channel connecting the two are normally closed, meaning no current will flow from source to drain. The gate is then responsible for opening the channel and letting current flow from source to drain. In a graphene field effect transistor this conductive (GFET) channel is made of graphene.

Biorecognition using GFETs works through the modulation of the graphene channel conductance. A change in the channel conductance will lead to a change in the measured source-drain (S/D) current. The channel conductance will change if charged particles are in close proximity to the graphene bridge (within the Debye Length). If the charged particles are within the Debye Length, the electric field from the particles will interfere with the graphene field effect and thereby change the channel conductance. This change in channel conductance can be quantified by changing the gate voltage over time, also known as sweeping, and thus, find the charge neutrality point also called Dirac point. This feature can be used for sensing of biomarkers in our case IL-6 because a change in (S/D) current and therefore a shift of the Dirac point is directly related to the presence of the biomarkers and their concentration as those have a negative charge in the case of IL-6.

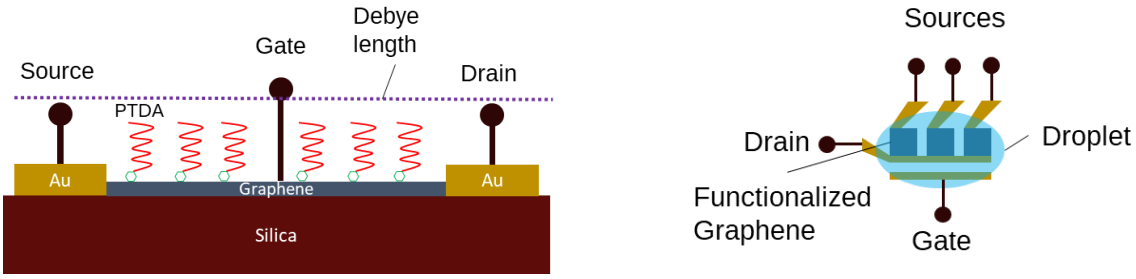


Figure 1. A schematic illustration of the functionalized GFETs with Pyrene-Tagged DNA Aptamers (PTDA) (cross sectional view) (left). A schematic illustration of the functionalized GFETs chip (top view) (right).

2.2 Molecular Recognition and Assay Reagents

Being able to only measure the specific negative charge of IL-6 and no other ions in the blood plasma sample of the patients is done by functionalizing the graphene bridge of the GFET with aptamers with high affinity to human IL-6 proteins. To additionally avoid any van der Walls interactions between the graphene and all sorts of molecules, the free spots not functionalized by the aptamers are covered with BSA.

Functionalizing the graphene on the GFET is done by pi-stacking interactions of an anchoring molecule, pyrene butyric acid N-succinimide ester (PBASE), that is connected covalently to the 5' aminated end of the IL-6 aptamer as can be seen in the Figure 2. When IL-6 binds to the aptamer the latter changes conformation due to this interaction, allowing for the negative charge of IL-6 to come closer to the graphene and therefore change the (S/D) current as explained above. Using PBASE as an anchoring molecule also allows to functionalize the GFET with different aptamers with affinity to other biomarkers.

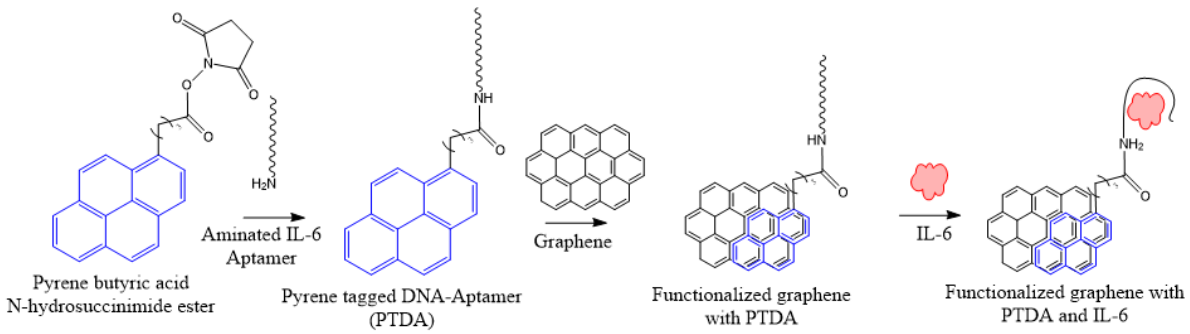


Figure 2. Schematic illustration of the steps of functionalizing the graphene on the GFET.

2.3 Cartridge Technology and Reader Instrument

The drain of the GFET device creates a relatively low current proportional to the amount of IL-6 on the device. This current and therefore the shift of the Dirac point is measured by a custom designed reading instrument. This reading instrument has the objective of reading the signal from the GFET and displaying the result on the Raspberry Pi 4. In other words, the reading instrument is a data acquisition system.

Figure 3. Simplified diagram of the measurement of the GFET's current by a custom-designed reading instrument with a further display of the results on Raspberry Pi 4.

This data acquisition system consists of three parts: Transimpedance Amplifier (TIA), Low pass filter (LPF) and Analog-to-Digital Converter (ADC).
The TIA first converts the current into a voltage and then amplifies in the range of 1 to 5 Volts. Then, this voltage is sent through the LPF, which filters out any noise. Lastly, the ADC converts this filtered voltage into a digital signal that can be read by the Raspberry Pi.

Figure 4. Illustration of the three parts of the data acquisition system (TIA, LPF, ADC).

2.4 User Interaction

The user will operate the instrument illustrated below to measure the concentration of IL-6. The operation will take 5 steps:

1. In the touchscreen, add or select the measurement ID;
2. Select "Background" for a background gate sweep;
3. When "Background Measurements Completed" is displayed on the screen, a drop of approx. 5 µL of plasma can be applied onto the chip;
4. Select "IL- Measurement". The instrument will sweep the voltage of the chip within the appropriate range;
5. When "IL-6 Measurements Completed" is displayed on the screen, the estimated IL-6 concentration will be displayed in the top right corner.

3 Technological feasibility

3.1 Physical transduction

The core part of our biosensor is the fabrication of our own chips in the cleanroom. Our current design is shown in Figure 5 Figure 5and consists of 18 GFETs, 2 source electrodes and 18 drain electrodes allowing us to still be flexible on which connections works the best.
This fabrication is correlated with many challenges. Challenges we are currently facing in the cleanroom are for example severe graphene delamination during UV development of photoresist. The problem can be seen in Figure 10 in the appendix (top left). Here graphene was passivated with Aluminum (Aluminum on top of graphene) and it is clearly seen that some Aluminum squares (and graphene squares) are missing.
After making appropriate changes to the process, this delamination is no longer a big issue as can be seen in the Figure 10 (bottom center). The figure shows the individual drain electrodes, the shared source and gate electrode. A microscope image of one source-drain connection at a higher magnification can be seen in Figure 10 in the appendix above (top right). Here, even at the increased magnification, the delamination is barely noticeable. However, we still have some issues to solve before we can make working GFETs. Therefore, at the competition we are using commercial GFETs fully sponsored by Graphenea.

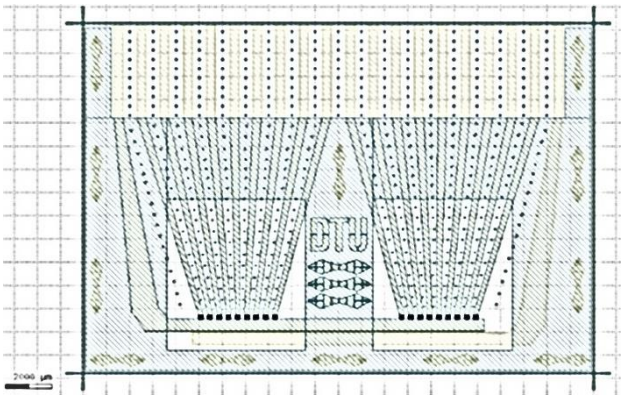


Figure 5. Design of our own chip.

3.2 Molecular recognition

In the development of our biosensor, the first step was to verify that the reaction between the anchoring molecule PBASE and the human IL-6 aptamer was successful. This was done with two different measurements. With a UV spectrophotometer we checked if the aptamers had been purified correctly after the reaction and were not washed away. Figure 6 shows the UV spectrum of PTDA and shows a peak at around 260 nm with a resulting concentration of 0.2 mM. The theoretical concentration being 0.245 mM this shows that aptamers were still in solution and only about 15% were lost during the washing process.

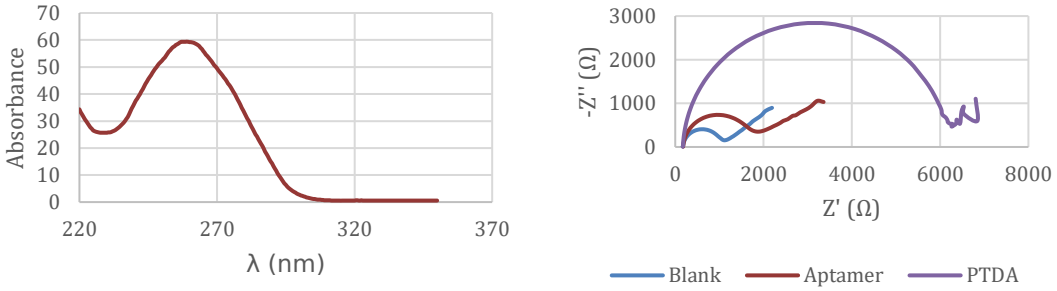


Figure 6. UV-Vis spectrum of the pyrene tagged DNA aptamer with a characteristic peak at 260 nm (left). Electrochemical impedance spectroscopy measurements (right).

To confirm that the aptamers are bound to the anchoring molecule, electrochemical impedance measurements were carried out. PTDA was functionalized on a graphite (multilayer of graphene) chip and the resistance was measured. Figure 6 shows that the resistance increased comparing only

aptamers that interact via van der Walls interaction with the graphite and PTDA that interact via stronger pi-interactions with the graphite.
KD, of aptamers to IL-6, was measured to be $19 \pm 4{,}5$nM as per manufacturer (BasePair Biotechnologies, Inc.) via microscale thermophoresis analysis [2].
In a second step the GFET's were evaluated for IL-6 detection. The electrical circuit is closed by adding a droplet over the GFET, which is also called top gating. The gate voltage at the charge neutrality point (Dirac Point) for different solutions can be measured. A blank measurement with only water shows a Dirac point a 1000mV as can be seen in Figure 7. Functionalizing the graphene with PTDA results in a shift of the Dirac point to lower voltages as the negative charges in the chore chain of the aptamers change the conductance. By adding IL-6 with two different concentrations on the chip and washing of the proteins that did not interact with the aptamers, the Dirac voltage decreases again for both concentrations respectively to about 740mV and 620mV. To determine the exact concentrations of IL-6 in plasma a calibration curve still has to be done.

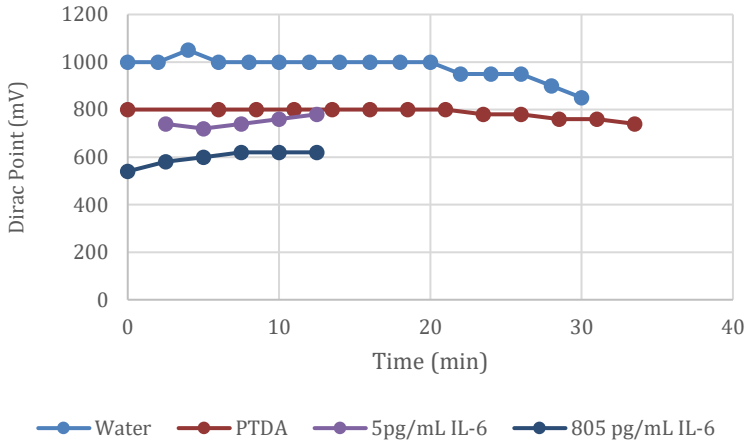


Figure 7. Evaluation of the GFETs for IL-6 detection by observing the Dirac voltage variations.

3.3 Cartridge

Ideally our cartridge consists of just one functionalized GFET chip as represented in Figure 5, at the moment, due to difficulties in fabrication, we are using commercially available chips provided by Graphenea.

3.4 Reader instrument

Figure 8 shows the construction of the device. A 7" touchscreen is used for control and interaction and all components are put in a compact 3D printed case.

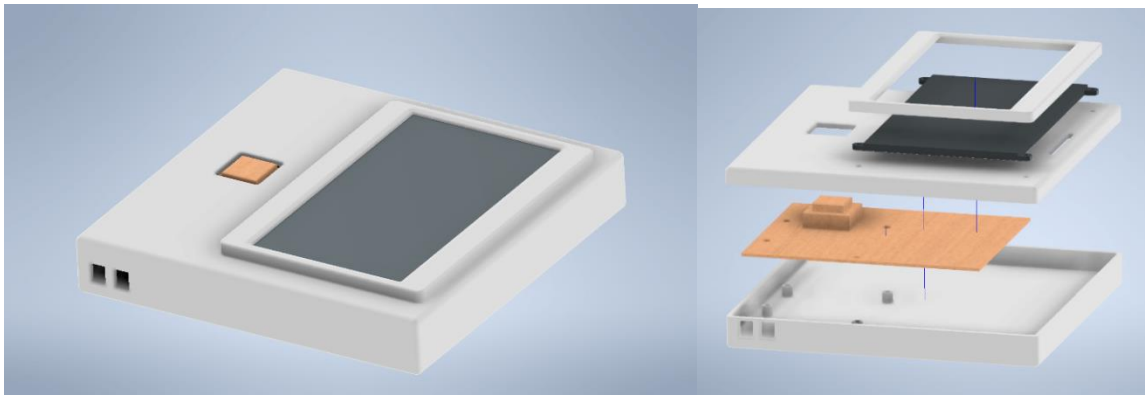


Figure 8. The assembled device (left). The main parts of the device from bottom to top: bottom part of the case, PCB with GFET chip and holder, top part of the case, touch screen, screen cover (right).

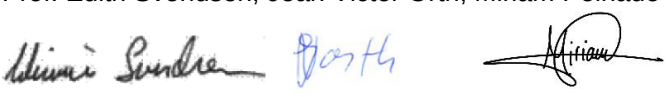
4 Originality

4.1 Team

Our novel graphene-based field effect transistor has multiple advantages, ranging from properties of 2D geometries like high sensitivity and easy integration capabilities to high carrier mobility. The possibility to change the gate voltage and therefore see a change in the charge neutrality point allows for an ultra-sensitive detection. [3] [4] [5]
Using aptamers as biorecognition sites is very advantageous in comparison to antibodies as the charge of the biomarker is closer to the GFET and therefore shows a higher signal. In addition to that, aptamers are cheaper in production than antibodies. The anchoring of the biorecognition molecule (in this case the aptamer) is done by pi-interaction with a pyrene tagged DNA aptamer (Figure 2) which allows to use various biorecognition molecules that could detect several biomarkers occurring in sepsis. [3] [6]
Our team worked this year on conceiving our own GFET in the cleanroom, making the functionalization with PTDA's on the chips and connecting them to our own device, so as not to rely on any provider from outside. As the fabrication of the chips in the cleanroom is very arduous in so little amount of time, we received a sponsorship of GFET chips from Graphenea for the competition and will continue working on our own chips in the future. [7]

4.2 Team supervisor

The DeTectUs team at DTU has worked on the development of a biosensor based on a graphene field effect transistor. The team came up with the idea by themselves and quickly contacted an expert on graphene to acquire more information about the material and how such a device could be built. The field is very novel and there is not that much literature on the subject. Difficulties involve device fabrication, as graphene is not very easy to work with, as well as device functionalization, as graphene has very few defects and is therefore not easily functionalized.
Both challenges were taken up by the team. Three of the students focused on the fabrication of the devices, trying out and optimizing a lot of processing steps in order to achieve the designed structures. Meanwhile another team of students focused on the functionalization, which involved attaching an aptamer towards IL-6 onto the graphene using pyrene and EDC/NHS as linkers. The students developed and optimized the protocols themselves with little help, based on the few journal articles that were available. The students selected the option of the aptamers independently, as they judged it was the most likely molecule to achieve results for the particular biosensor they were going to develop.
A third team worked on the electronics development. Apart from an initial talk with some of the DeTectUs members from the last few years, the team has developed their prototype by themselves, without any input. A fourth team has been looking at finding sponsors, talking with companies, doctors and other business specialists, for the most part people they themselves had researched and found.
All in all, this year's team has a truly novel idea and they have developed it and optimized it without significant outside contribution.
Prof. Edith Svendsen, Jean Victor Orth, Miriam Peinado Martin



5 Translation potential

5.1 Business Model Canvas

Figure 9. Business Model Canvas.

5.2 Market Description:

Everybody has heard about COVID-19 pandemic and the reasons behind the world trapped in lockdown. If we look closely at the deaths related to COVID, the data from the US says that sepsis was the cause of every 1 out of 3 covid related deaths [8]. There are around 49 million cases of sepsis around the world, and 80% of those cases are found in children and people above the age of 60. This data is recorded only in hospitals and ICUs which is not very representative of the overall tally, and this means many more cases exist. Out of those 49 million cases of Sepsis there are 11 million deaths, and one of the major reasons is multiple organ failure because of the late diagnosis of Sepsis. The complete recovery rate from sepsis is only around 50% while the remaining either die from sepsis within a year or get-affected by long term disabilities. [9] [10]

Currently, patients with undiagnosed symptoms of sepsis that go to a doctor have to go through a series of tests including x-ray, blood tests, biomarker identification and testing for organ failure. After diagnosing sepsis, doctors must identify which bacteria or virus is affecting the person. This complete diagnosis of sepsis can take up to 5 days [11] as shown in case persona in section 9.2. This method shown in Figure 11 is slow, expensive, time-consuming, and not available 24/7 everywhere. Our solution is based on making a point of care (POC) device which will integrate with current methods of detecting sepsis and could be tied into existing systems used in various hospital ICUs, ER ecosystem and ambulances. Our device will be regulated by (EU MDR) 2017/745 and (EU IVDR) 2017/746 regulations for EU countries including Denmark. This regulation classifies a biosensor like the one created by our team as a Class I medical device, and as such, it must adhere to its regulatory procedures. The product must undergo homologation and obtain the CE certification to be commercialized in the medical device market.

5.3 Stakeholder desirability

After meeting with the UK, French and Belgium Sepsis trust, we found three major pain points in the system. The first one is insufficient facilities to test for sepsis since it takes a lot of time, money, and government approvals for implementing the machines in the ICU setting, and trained medical professionals are needed for using those devices. The second problem is mentioned by Ron Daniels

from UK Sepsis trust: “the current tests are done by medically trained professionals and not easily available in rural regions” [11]; Prof. Djilani from a hospital in France mentions: “My lab is running 24/7 for the blood test related for sepsis and many hospitals lack these facilities.” [12] The third issue is identified by Belgium sepsis trust: “Belgium does not have a protocol regarding sepsis detection. It is difficult to bring back doctors off duty when patients with sepsis symptoms comes in. A model which could take the readings and can probabilistically say that a patient has chances of sepsis or not will be better for the doctor to react as quick as possible.” - Belgium sepsis trust [13].
These challenges faced by our secondary stakeholders in treating our primary stakeholder (people affected by sepsis) are crucial to address. While medical professionals are aiming to get very accurate results by sending probes to laboratories, a large amount of time could be saved by having standard sepsis detecting devices in hospitals, ICU’s or even ambulances.
Our biosensor is a graphene field effect transistor capable of rapidly measuring low concentrations of 3-4 different biomarkers in a patient’s blood at the same time, thus solving all three major problems mentioned by health care professionals and organizations. By implementing our biosensor at different hospitals and clinics, a simple blood sample will speed up the detection of Sepsis. The data generated from the test will be sent back into the system for continuous improvement of the model accuracy. This will be done after ensuring the proper data safety laws stated according to Europe and US standards. The handy medical device will not only give the result in less than 15 minutes, which saves up to 1 day in the testing, but also reduce the expenses for training of professionals. The DeTectUs device is adaptable and multifaceted, thus offering high scalability. The same G-FET can be used to detect other biomarkers which change during Sepsis and Septic shock. Therefore, our G-FET platform will improve the biomarker detection system required for Sepsis and pivot towards detection of different diseases. We envision that our biosensor will be key in fighting sepsis across the world and fill the gaps mentioned in the current medical system.

5.4 Business Feasibility

In this business feasibility study, we describe the required resources as well as strategies needed to commercialize our product as a startup. Initially, in the team structure we will have a CEO, COO and engineers spearheading their respective departments. The business potential handling person will manage all the finances until the time we get a good financial manager on board. Initially, we will work in WS labs to improve our project along with expertise from Winnie Svendsen as one of our board members. We are in talks with Høiberg patent attorney regarding the tech transfer of our patent from university and filing multiple patents to protect our product, process, and its uses, which covers all possibilities. At the same time, we will pivot our project to larger scale in DTU start-up incubator and after we have our patent filed, we will apply for Accelerator program in Bio-Innovation Institute or Accelerance in Denmark towards growing fast and to getting our project running.
Initially, we plan to raise the soft funding from multiple grants across Denmark such as Mikrolægget, Innofund Denmark, Erhvervshus and Skylab fund. We have defined a timeline to raise 2-3 million Euros of seed-funding or angel investment round and afterwards in Series-A round a further 10-12 million Euros for reaching clinical trials including R&D and embedded systems in the device, hiring patent consultants, strategy consultants, clinical trial consultants and application for CE marking. We can raise funds from SofiNOVA Ventures, Rock Health, Novo Seeds. Our approach towards a GFET biosensor, helps us gain support from Radiometer with expertise in medical instruments, Graphenea with premade GFETs for proof of concept, Kayaku with materials and knowledge for our own GFETs, BasePair Biotechnologies with aptamers alongside Knud Højgaards Fund, and Otto Mønsteds Fund for travel expenses to events and conferences. The R&D phase with clinical studies will be completed in 4 years and we will hit the market in the 5th year entering first in Germany followed by Scandinavia (Denmark, Sweden, Norway), France, UK, Belgium and followed by Spain and Italy. We have collaborated with UK Sepsis Trust, French Sepsis Trust and Belgium Sepsis Trust and in talks with Swedish Sepsis trust which are expanding in Norway and Denmark. We will also help hospital ecosystems in implementing our platform-based technology in their existing system with proper cost-analysis for implementing our biosensor. By 2030 we will enter the US market, and our choice of country goes as per the GDP per

capita of the country to make our start-up profitable. Our biosensor will save approx. 1 day which translates to 1,230 Euro with almost 1000 Euro of Medicines, this could be depicted from Figure 12 in the appendix. This lucrative saving will help us collaborate with insurance companies and State services.
We will use the razor-blade business model for our GFETs and will supply it with multiple channels such as tenders for Government orders, medical suppliers, direct selling to the private testing labs – all being B2B. Associating with Sepsis Trust will help us improve our technology, getting in touch with Governments to understand their perspective and pitching our solution to them. Regarding partnership, all the quality checks will be done by our company and by forming key partnerships with Radiometer, BasePair and Kayaku we outsource manufacturing and assembling in each country as per requirements.

5.5 Financial Viability

We have gathered information and data from our business advisors, financial stakeholders and medical suppliers based on the survey we conducted to create a viable business model. Currently, we have thought of using the razor-blade business model and are ready to adapt as per the needs of the market. After a cost study, assumption of sales and revenue models of our product, we will base our R&D department, business department based in Denmark until the time we reach the team of 40-50 engineers. After that point we will shift our base to Switzerland, these strategies been used by multiple start-ups to save major money in terms of revenue. There will be centralized production center in Germany or Poland based on the cost-economic accessibility of setting up the industry. The Instrument will cost about € 16,000 (including the embedded system) and the chips will cost € 300. The service regarding the device will be included in the package. The pricing of the chip is based on value-based pricing and cost-based pricing.
Taking into account various things that can affect the market such as ordinary cases of Sepsis, which comes in hospital, continuous monitoring post surgeries and in case of pandemic situation we have made cash flow and profit estimations. We expect a profit of about € 6 million per 365,000 tests annually, based on reaching around 1% of the yearly medical consultations done on Sepsis in ordinary market. We expect that every machine will test on average 20 tests per day (including busy zone and non-busy zone). Our aim is to deploy approx. 55 machines around major hospitals and big cities. By the end of 2029 covering the European countries and then targeting the US, Canada, and China market.
We estimate that in our first year we will raise around € 70,000 in soft funding from various organization foundations in Denmark, which will help us generate good data and file the patent. In the second year, we plan to raise around € 4 million towards making our first prototype and allowing expansion strategies line-up for regulatory, clinical trials and partnerships. In the third year we will aim to raise almost € 11 million for conducting clinical trials and generation of models. It will help in gaining the patent and the confidence of the people. Figure 13 depicts the financial revenue trend for the initial years of our company based on the estimations and calculations. As mentioned, we will secure another series A funding of about €11 million for the completion phase of clinical trial and for the launch of our product. With this in 10 years, initial funding will be returned by 10-15 times the amount invested. In the event of any major disease outbreak, our sales will shoot up as the chances of organ failure increase in most health conditions. If we capture 2% of the market, then our device will generate around € 100 million revenue while saving more than € 300 million for Government and insurance companies combined and saving thousands of lives. We aim to capture at least around 10% of the market by the end of 2033. The response to our current approach makes us open to partnership and in a good position for getting acquired by big MedTech companies or opportunities for merger.

6 Team and support

6.1 Contribution of the team members

Jean Victor Orth	Team captain directing the team to a common goal and part of the BioChem team. Has been actively involved in the validation and testing of our biosensor. He also scored us a sponsorship from Basepair.
Miriam Peinado Martin	Team captain, and in charge of our social media and other creativities. Miriam is furthermore part of the Electronics team and took an active role in the development of the business plan. Miriam, has also scored us the sponsorship from Graphenea.
Yashomangalam Bhutada	Part of the Management team. He opened many doors for us in terms of partners, sponsors and meeting sepsis association representatives and VOC from around Europe. He oversaw the business and entrepreneurship sides of the project.
Adrian Nastas	Part of the BioChem team and has been our spokesperson in contact with Radiometer, who have help us both financially and with knowledge. Has also worked on the validation and testing of the biosensor.
Marlene Antunes	Part of the Electronics and Activities teams. Has had an important role in making electronics of the biosensor work. Helped in the testing and calibration of the biosensor. Marlene has also been involved in co-organizing team building activities.
Junaid Qazi	Part of the Electronics team. Has been heavily involved in all the electronics and embedded systems aspects of the project. He has designed all the necessary PCBs and the biosensor testing environment.
Anna Guitó Vilardell	Part of the BioChem and Activities teams. Has helped with making all the necessary solutions for the functionalization and validation of the chemical principles behind our detection method. Anna, has also organized multiple team-building activities.
André Fontes	Part of the BioChem. Has helped with the preparation of solutions necessary for the functionalization of the biosensor.
Celine Schou Dinesen	Part of the Cleanroom and Activities teams. Has supported the cleanroom fabrication of our own chips. Celine was also involved in co-organizing team building activities.
Abel Botond Vamosi	Part of the Electronics team. Has worked on and helped with the design of the device.
Erkan Karataş	Part of the Cleanroom team. Has worked on the design and fabrication of our own chip. Has also been a link to the Physics department at DTU, who have helped with knowledge and experience on FET and graphene.
Ömer Altan	Part of the Cleanroom team. Has worked on the fabrication of our chips.
Sanjith Krishna	Part of the BioChem team. Helped prepare solutions necessary for functionalizing of the biosensor and has also contributed on developing the business plan.
Mihaela Georgescu	Part of the BioChem team. Has helped with the preparation of solutions necessary for the functionalization of the biosensor.

6.2 People who have given support

We would like to sincerely thank the following people for their constant support on the scientific, entrepreneurial, organizational and creative parts of the project:
Winnie Edith Svendsen (DTU), Maria Dimaki (DTU), Christian Vinther Bertelsen (DTU), Gitte Elvers-Thomsen (DTU), Peter Bøggild (DTU), Tim Booth (DTU), Thiago Sousa (DTU), Abhay Shivayogimath (DTU), Jie Ji (DTU), Yingqiu Zhou (DTU), Manh-Ha Doan (DTU), Sarah W. Anker (DTU), Lasse Nørregaard (XY Therapeutics, BOOST Pharma), Rajat Mehta (Radiometer), Allan Byrnard (Radiometer), Alba Centeno (Graphenea), Elias Torres (Graphenea), Jesus de la Fuente (Graphenea), Alex Chiu (Basepair), Ron Daniels (UK Sepsis Trust), Djillali Annane (France Sepsis Association), Jamila Hedjal (France Sepsis Association), Carine Nielsen (Sepsibel), Michael Clarke (Sepsibel).

6.3 Sponsors

BasePair Biotechnologies	Provided aptamers to be immobilized on sensor surface.
Graphenea	Supported with knowledge and feedback for our cleanroom process. Sponsored around 80 BioFet chips of their own.
Radiometer	Funded money for electronics and merchandise.
Knud Højgaards Fond	Funded money for travel expenses.
Otto Mønsteds Fond	Funded money for travel expenses.
Blue Dot (DTU)	Funded money for materials and others.

7 Final Remarks

As engineers we want to know the inside nature of things and to continuously improve and develop ourselves, hence pursuing ever new ideas. However, we should not forget to finish the projects that we have already started. With this project, we feel the responsibility to continue working the problem of detecting sepsis, which has become personal to us. Thus, our future plans would be to develop our biosensor idea further, working on optimizing and improving the current results. As the detection method we use is flexible and precise it can provide fast and cheap answers for other conditions and diseases. The next step would be to jointly unite into a start-up and seek investments. Like this we seek to improve people's standards of living not only by saving lives but also by contributing to the EU economy.

We consider that we have learned a lot and that this competition has opened a lot of doors for us not only in terms of opportunities but most importantly in terms of knowledge and people that we have met, to all of whom we are very grateful. We would also like to thank the SensUs organization for making this competition possible. Furthermore, we look forward to all the feedback we will receive at the SensUs Innovation Days.

8 References

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9 Appendix

9.1 Cleanroom fabrication

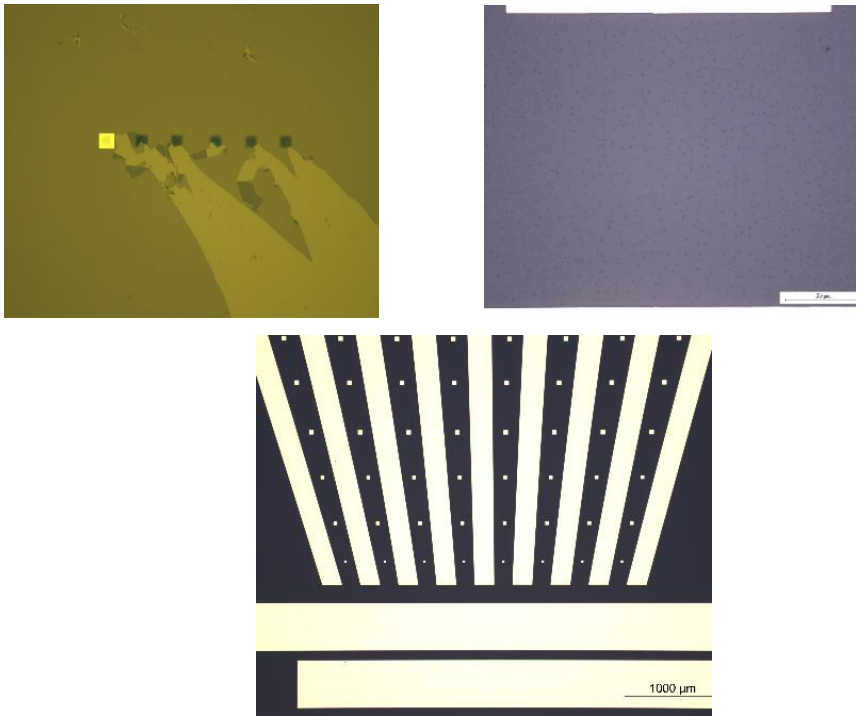

Figure 10. Graphene delamination during UV development of photoresist (top left). A microscope image of one source-drain connection at a higher magnification showing a graphene layer with no damage nor impurities (top right). Picture of our current chip which is in development at this moment (bottom center).

9.2 Case persona

Marcus is 65 years with Chronic Obstructive Pulmonary Disease (COPD), fatigue, diarrhea. It comes to hospital for treatment. The doctor starts with diagnosis of Sepsis.

The steps of Sepsis diagnosis are as follows:

1. Chest X-ray: To check for pneumonia
2. BNP measurement: Normal reading ≤ 100; rule out heart failure
3. D-Dimer measurement: Elevated D-dimer measurement strongly related with organ dysfunction
4. WBC count, abnormal if >12,000/mm3 or <4,000/mm3
5. Biological signs of information (for ruling out bacterial sepsis)
    a. CRP
    b. Procalcitonin
    c. Lactate
    d. TCP
6. Blood culture to check which bacteria is causing sepsis (result can take up to 1-2 days). Because of not having access to the patient's lungs.

The abovementioned process can take from 1-5 days for detection of sepsis, depending upon the location and the facility available in the nearest testing lab.

9.3 Disease Management Timeline for Sepsis and Septic Shock

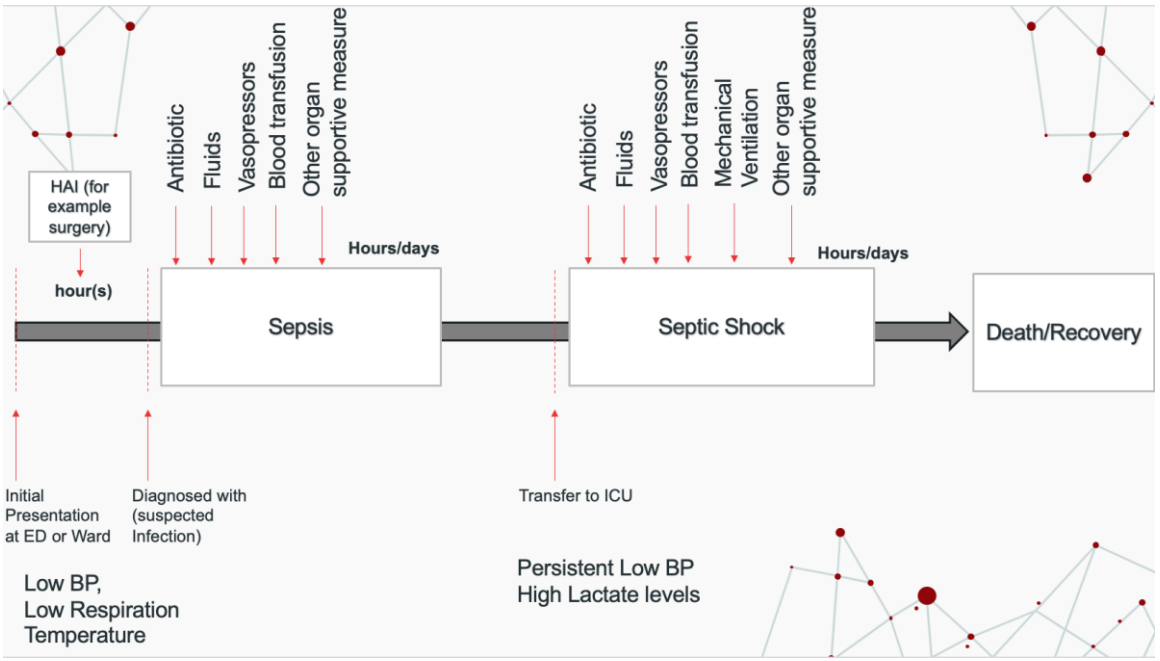

Figure 11. Diagram of the disease management timeline for Sepsis and Septic shock.

9.4 Cost Analysis

DeTectUs Solution

	G-FET # chips	G-FET Price (€)	Chemicals Quantity (ug)	Chemicals Price ($)	Chemicals Price (€)
	16	€ 300.00	100	$ 50.00	€ 49.11
needs for 1 measurement	1	€ **18.75**	20	$ 3.00	€ **2.95**

Human cost		
monthly salary	40,000.00 DKK	
price/hour	250.00 DKK	
125 chips / week	37h	9,250.00 DKK
price/chip	74.00 DKK	**9.87 €**

Shipment*
€ **30.00**

Assumptions:	
1	We will need 3-4 biomarkers to be able to probablistically confirm or deny a sepsis diagnosis via model created
2	DeTectUs biosensor package for sepsis will include: 4 chips for the 4 biomarkers measurements, embedded system for notification, results and shipping
3	The machine price is assumed

*Shipment is asumed to be european

	Creation cost	Partner's cost	Market cost	
DeTectUs pricing / package:	€ 156.25	€ 312.50	€ 351.57	Selling via multiple channel
DeTectUs pricing / machine:	€ 8,000.00	€ 16,000.00	€ 18,000.00	

European Market as today

cost / patient with sepsis (Europe)	16,000.00 €

Avg. time of patient (days)	Avg. cost per patient	
14	16,000.00 €	
1	1,142.86 €	
2.5	2,857.14 €	*See assumption 4*

Assumptions:	
4	Equal amount of money spent throughout 14 days
5	takes between 1-5 days to diagnose sepsis; we will assume an average of 2,5 days*
6	Using DeTectUs solution diagnose can be done 1 day earlier
7	Capturing 2% of the Europe's Market
8	Every 7 in 10 patient is testing positive

*based on interviews to doctors

Total number of diagnosed sepsis cases in Europe:	3,400,000	
Amount of patient (SIRS symptoms)	0.70	*Based on interview with Ron Daniels*
Total Market consideting people testing negative	14,571,429	*See asumption 7*
Target Addressable market (Europe)	4,857,143	
Captured market (2% of total)	291,429	patients / year

Amount of Money saved a year using DeTectUs biosensor	333,061,224.49 €

Number of testing kit require to do the test		*1*
	Revenue per chip:	€ 312.50

No. of our testing set require to do the test in 1 day	1		
Revenue generated from chip sets per patient	€ 312.50	Euro	
Expected Annual Revenue of DetectUs in 5-7 years	€ 91,072,516.57	Euro	The revenue generated by our company from chips is 100 Million

Figure 12. Pricing of our chip meant to be used in the biosensor.

9.5 Financial structure

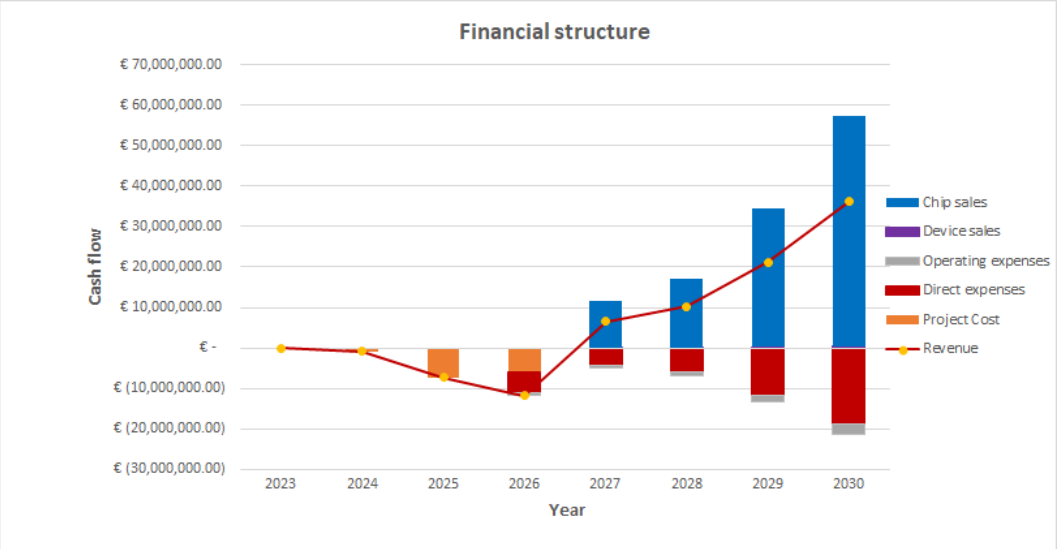

Figure 13. Revenue structure over 8 years.