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1 Summary for SensUs

1% of the entire world population suffers from Rheumatoid Arthritis. To alleviate symptoms patients are given adalimumab, which is a powerful drug known to cause problematic side effects. At the same time, immunity towards the drug can develop in the patient over time, making the treatment inefficient, as well as creating obstacles for the patients' care trajectory. To reduce the occurrence of these issues, we suggest monitoring the concentration of adalimumab, as well as the formation of antibodies, in patients and correlate this to immunity of the drug as well as treatment efficiency.

We created a system to determine the concentration of adalimumab in plasma. The principle used is the surface plasmon resonance (SPR). In this technology the refractive index of the sample is measured, through an angle change, which we then correlate to a calibration curve, responding to a specific concentration of adalimumab. Depending on the concentration of adalimumab, the refractive index of the sample changes.

The biosensing part includes the gold surface modification with a linker type molecule and an engineered antigen against adalimumab on top. This system is capable of capturing adalimumab which is then detected by SPR.

2 Biosensor System and Assay

2.1 Molecular recognition and assay reagents

For the molecular recognition of adalimumab, four peptides mimicking the site of interaction of TNF alpha with adalimumab were engineered.[1] Additional modifications were made to enhance the immobilization of the peptides to the sensor surface. The affinity of TNF alpha and these peptides against adalimumab were tested by ELISA and compared. As the results of two of the peptides were promising, it was decided to continue with them as potential epitopes for the sensor.

The gold surface of the sensor was functionalized with a self-assembled monolayer composed of 3-Mercaptopropionic acid (MPA) [2]. After activation of the carboxylic acid of MPA with N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and N-Hydroxysuccinimide (NHS), the peptide was attached via an amide bond [3]. The free carboxylic acid residues were blocked with ethanolamine before the surfaces were ready to use in the sensor setup. The composition of the surface is shown in Figure 1.

2.2 Physical transduction

SPR are oscillating electromagnetic waves of conduction electrons on the interface between a metal and dielectric. The conduction electrons have a resonant frequency at which they are excited leading to these oscillations. As resonance is a requirement only certain energies can excite the plasmons. This is done by matching the momentum of a Transverse Magnetic oscillation (polarised light), to the oscillation of the plasmons [4]. When this coupling happens SPR waves propagate along the surface of the metal film, gold in this case, and exponentially decays parallel to the surface [5].

As light coming from the medium of air cannot match the momentum of the plasmons by itself, the SPR is only possible with a coupling mechanism: using a higher refractive index material on one side of the metal than the other. Prisms are commonly used as they have this higher index and a found in several shapes.

In this project, the Kretschmann configuration is used, as seen in Figure 1. The principle can be shown mathematically as:

$$k_0 n_p \sin(\theta) = k_0 \sqrt{\frac{\epsilon_{mr} n_a^2}{\epsilon_{mr} + n_a^2}}$$

Where, n_a is the refractive index of the analyte, ϵ_{mr} is the real part of the dielectric constant of the material, n_p is the refractive index of the prism, θ is the angle of incidence on the metal layer and k_0 is the free space wavenumber of the optical wave. It can be seen that only certain angles of the incoming light matches the resonance condition [4].

A different concentration of adalimumab means a change in the n_a which leads to a change in SPR excitation angle.

Here it can be seen that optimisation of the optical setup can be done using several parameters, such as the metal layer, the RI of the coupling materials (prism and substrate), focal length of focusing and collimating lens, and also the length between the CCD detector and the final collimating lens.

2.3 Cartridge technology

Currently there is no cartridge system. Our chip will be placed directly on the prism by hand. And the plasma will be put on manually as well.

2.4 Reader instrument and user interaction

A custom setup has been made due to being able to make small increments in the angle to optimize the system. The setup has been made in aluminium due to the combination of stiffness and low weight. The Setup currently measures 470x300x100 mm and can change the angle of the camera and laser from 35-85 degrees, due to the earlier need to both measure in water and air. If the setup was made now the size could therefore be reduced, by lessening the range of the setup. The

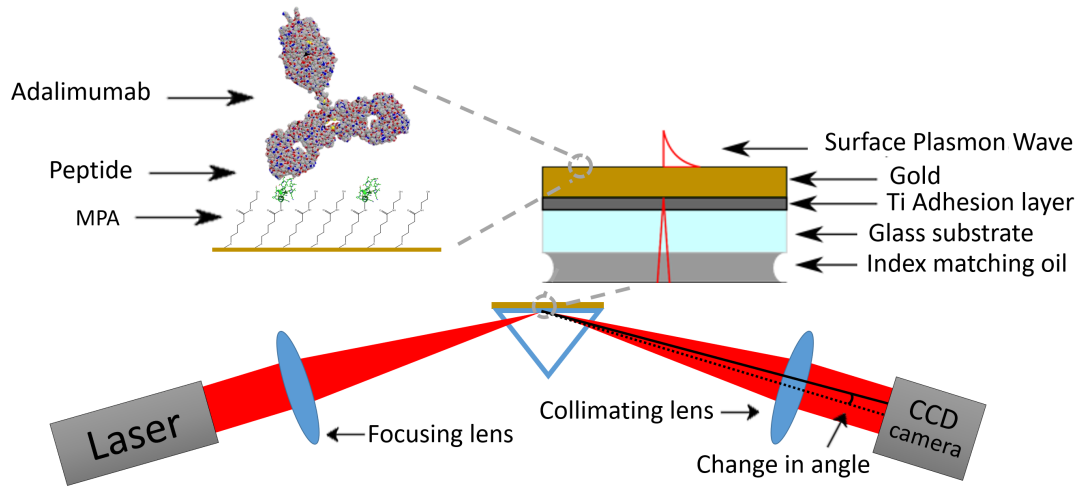


Figure 1: Figure showing the Kretschmann configuration of an SPR setup, where light is hitting the gold film at the angle where SPR happens. Moreover, a schematic of the surface functionalization is shown.

setup uses thread and bolts to change the angle of the setup. The reason for this is two-fold. The thread and bolt has a self-locking ability when sufficient axial load is applied. Secondly the axial load on the bolt will at a low cost also minimize slack in the system which allows for dependably to change the setup with small steps. Due to using a screw and thread to change the angle there is practically no slack in the system. This results in the system being more stable and being able to do smaller step increments than using commercial holders. The increment depends on the current angle of the system, with the highest increment being 0.4° . Due to the aforementioned minimal amount of slack increments of 0.1° is possible.

In the current design the user would have to pipette the plasma on the chip. To increase accuracy the user could create an turbulent environment for the plasma, by repeatedly pipetting the plasma in and out of the pipette. Then the chip can be put on the prism to measure the concentration of adalimumab.

2.5 Dataprocessing

To detect the angle change of the laser an analog one-dimensional CCD camera is utilized. The data from the one-dimensional camera is interpreted by an STM32 Nucleo-64 development board. Here the on-chip ADC for readings was utilized and various pins to drive the CCD camera. The STM32 was chosen for its high timing frequencies and ADC sampling rates.

The microcontroller takes a sample picture before and after the sample has been added and calculates the difference in angles using an algorithm. The change in angle is compared to a calibration curve, and an estimate of the concentration of adalimumab is finally displayed on an LCD or a connected computer through USB.

The software is custom written by the team but uses general functions for the setup of the various communications for the microcontroller (I2C, Uart, ADC-control). All the calculations are performed automatically when the system is turned on.

3 Novelty and Creativity

3.1 Already available

The fabrication of the self assembled monolayer with MPA attached to a gold surface as linker molecule is a well established method [3] and was used to immobilize the epitope on the sensor surface. For the biological development of the system, access was granted to biological labs, ELISA readers, potentiostats, spectrophotometer and stock chemicals.

SPR has been shown as an successful method to detect adalimumab [6] and commercial working systems are already on the market. Access to an optical laboratory was granted. There, it was possible to find the main optical components needed to build the first prototype such as lenses, a prism, a light source and a CCD camera. The gold surfaces were fabricated in a cleanroom (DTU Nanolab) using electron beam evaporator technology and the wafers were diced using an automatic dicing saw.

To develop the reader instrument access to electrical labs, soldering equipment, function generators and oscilloscopes were given. Commercial micro-controllers and analogue CCD cameras were bought to digitize data for data-processing. A prototyping lab and material stock was also given access to, to build the setup.

3.2 New developments

The choice of a one-dimensional camera was selected since they are remarkably cheaper than two-dimensional CCD cameras (a difference of 2080€), and we managed to get sufficient data from the one-dimensional camera, to determine the desired concentration. At the same time the dataprocessing becomes greatly simplified with the reduced amount of data, resulting in simpler software.

The peptide "PepTeN1" that is used for adalimumab recognition was engineered by the team. The sequence was inspired by the binding site of TNF alpha towards adalimumab. By using a short peptide sequence instead of a whole protein, potential undesired non-specific interactions with other blood plasma compounds can be avoided. Furthermore, the affinity towards adalimumab is not dependent on the correct folding of the epitope. The unfolding can be disregarded due to the choice of a peptide as opposed to a protein, which is composed of a shorter amino acid chain.

From the physical transduction point of view the entire system was designed and built by ourselves. The prototypes had to be built from scratch, which for this purpose had not been done before. Thus, a working SPR detection system for the purpose of biosensing was built. Many parts of the optical holders were fabricated in a mechanical workshop with a precision of IT7-IT11 depending on the part. Using the self built holder and some commercial optical components such as lenses, the system was built from scratch.

4 Analytical Performance

4.1 Angle detection

The performance of the electrical system capable of detecting the angle change of plasmon lines is shown in Figure 2. The higher the voltage on the graphs, the lower the light intensity. A potential of 2.3 V corresponds to no light being registered by the pixel. The graphs illustrates that the 1D CCD camera is clearly capable of detecting changes in angles.

The change previously explained can be also shown as the beam spot of the laser. In figure 3 the dark band is the light that has been coupled into the plasmons and therefore is propagating in the surface. Depending on the concentration of adalimumab the dark line moves as it has been explained in section 2.2.

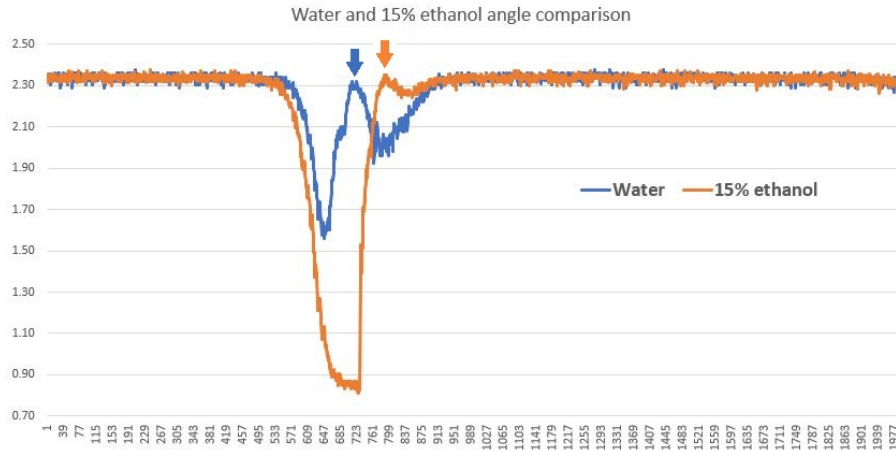


Figure 2: Intensity curves where potential as function of pixel number is plotted for pure water and water with 15% ethanol. The higher the value of voltage, the lower the intensity.

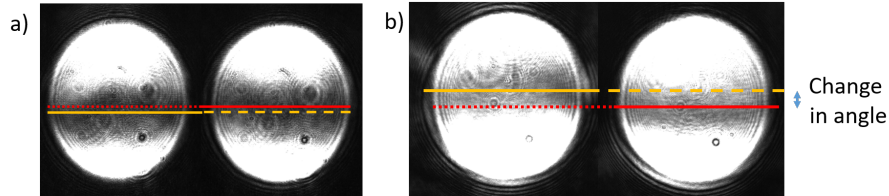


Figure 3: Images taken with a 2D CCD camera of the laser beam spot after interaction with the sample. The analytes are a) Left: buffer. Right: buffer and 10 μg adalimumab/mL. b) Left: plasma. Right: plasma and 10 μg adalimumab/mL. The yellow line indicates the excitation angle of buffer(plasma) and the red one the angle for buffer (plasma) and adalimumab.

5 Translational Potential

The translational potential and subsequent analyses have been conducted from an entrepreneurial perspective, and assuming we will launch a start-up company with our sensor within a reasonable timeframe.

The essence of the business model is based on the fact that it can be extremely troublesome to be treated with Adalimumab - the medicament Humira. It is especially difficult knowing if the drug is even going to work on the patient or if antibodies for TNF- α inhibitors, such as Humira have developed in the blood. Pre-screening before- and Therapeutic Drug Monitoring (TDM) while a patient is administered Humira is the solution to this!

5.1 Business model canvas

Figure 4 belows shows the business model canvas developed in DeTectUs.

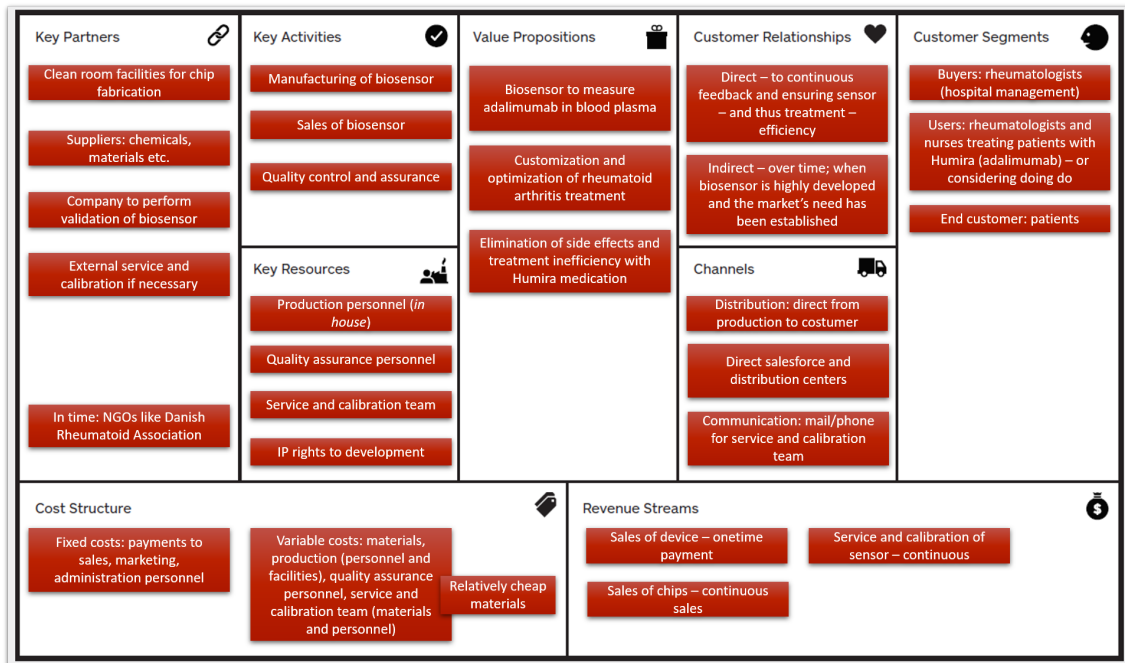


Figure 4: The business model canvas for the DeTectUs sensor.

5.2 Stakeholder desirability

A map of the main stakeholders can be seen in Figure 5. The faded boxes indicate stakeholders that are not relevant right now at this current phase of the project. As indicated, the most important stakeholders are of course the project owners: the DeTectUs students as well as supervisors - but equally important is the main customer - the hospital administration, who are the ones to make the final decision on purchasing the sensor. What's important to note is the fact that all the stakeholders' positions are dynamic and can change based on situation. For example are the potential sensor companies as customers not very relevant per se, but if the start-up suddenly has more traction in the sensor development industry in comparison to medicinal industry, those companies will change to be the main customer or partner and increase drastically in power.

An explanatory figure of what the different areas of the matrix means communication wise for the stakeholders, see Figure 7 in Appendix.

The Value Proposition shown in the Business Model Canvas is elaborated a little more in Figure 6. From the rheumatologists point of view, the primary pains, gains and corresponding gain creators and pain relievers are illustrated.

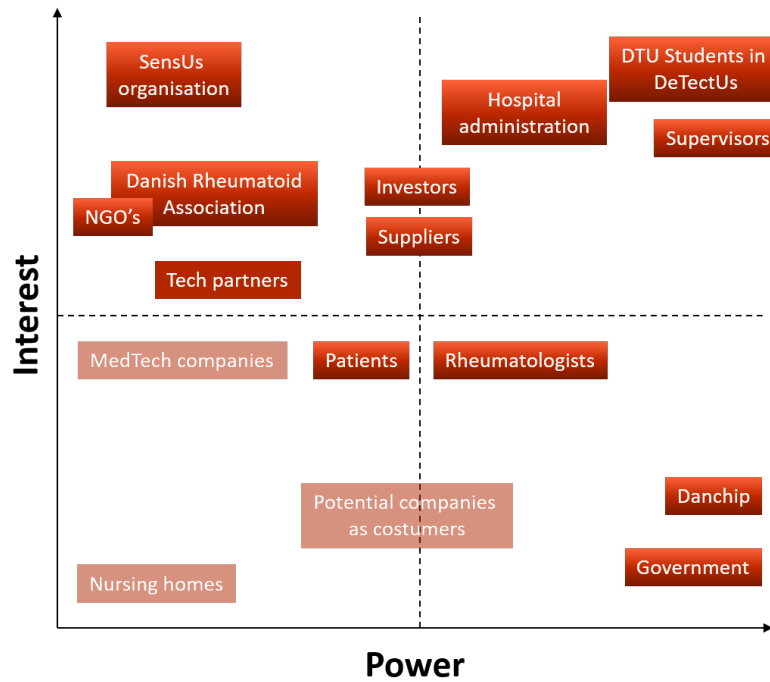


Figure 5: Overview of the most important stakeholders.

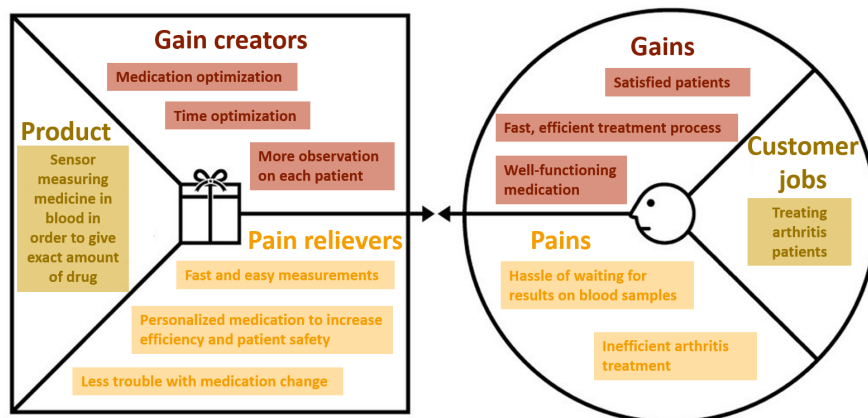


Figure 6: The value proposition map with the rheumatologists as the primary "customer" (user), as they would be the ones using the device. However note that the real customer is the hospital management deciding if money should be allocated for the sensor.

5.3 Financial viability

The sensor device itself is fairly cheap to produce. The most costly part of the sensor is the optical setup, amounting to about 450 € per sensor including all manufacturing. This is a one-time payment per rheumatology department, instead the use phase of the device should be taken into consideration with a price of approx. 1 €, which would have to be used every 8 weeks for each patient.

5.4 Business feasibility

DeTectUs was part of a startup accelerator at the innovation hub Skylab at DTU in the spring of 2019. Here, we gained valuable knowledge on business development and market insight, as well as

got constructive feedback on our analyses.

The business feasibility has been assessed partly through Porter's Five Forces in order to analyze the competitive framework for the sensor development. Figure 8 below shows where the main issues could potentially arise. There is currently no actual competition on the market, as the treatment framework we will be providing is an untapped market. In the primary phases of the product launch, we will be focusing on the market entry and the buyer power to ensure a balance in the scale up of production without compromising with the business model in Figure 4.

6 Team and Support

6.1 Contribution of the team members

Subteam	Responsibilities	Team members
Biotechnical team	Surface functionalization, Epitope design	Shreya Joshi Marina Nocera Dániel Berkes Shaghayegh Amirijavid Bettina Hierzberger
Nanotechnical team	Design and construction of the optical set up Optimization of chips and optical setup, data analysis of SPR images	Eva Zeqiraj Nuria del Castillo Nicolai Støvring
Entrepreneurship team	Business development and stakeholder management	Amalie Rasmussen
Product design team	Design and production of custom setup Graphical design	Luca Giannini Marc Johannes van der Zwan
Electrical team	PCB design, soldering, CCD camera integration, software, calibration	Thomas Frederiksen Berk Gezer

6.2 People who have given support

- Annette Hølek: Administrative supervisor, anhole@nanotech.dtu.dk
- Julio César Franco: Team coordinator, julfra@nanotech.dtu.dk
- Maria Dimaki: Academic supervisor, maria.dimaki@nanotech.dtu.dk
- Natalie Kostasheva: Technical supervisor, nako@nanotech.dtu.dk
- Jaime Castillo: Technical supervisor, jaic@dtu.dk
- Winnie Svendsen: Supervisor and contact person for the SensUs organization, winnie.svendsen@nanotech.dtu.dk
- Osamu Takayama: Photonics supervisor, otak@fotonik.dtu.dk
- Marie Sofie Møller: Adjunkt, Technical advisor on commercial SPR system, msmo@dtu.dk

6.3 Sponsors

6.3.1 Blue Dot Projects DTU



The DetectUs group belongs to the prestigious Blue Dot project portfolio of DTU. The Blue Dot Projects are projects that seek to increase awareness of sustainability, and launch real life research projects.

6.3.2 Gigtforeningen



The Danish rheumatism association hasn't sponsored us with any financial resources, but contributed with valuable insight, which has helped with validating our business models.

6.3.3 The Technology Partnership (TTP)



The Technology Partnership brought valuable setup optimization through a feedback session in the early stage of prototype development.

7 Final Remarks

In the spring of 2019 DetectUs was a part of a startup accelerator called Ignite at the innovation hub at DTU called Skylab. We would like to thank the whole team of Ignite for valuable market insight, feedback on value propositions, stakeholders analysis and business models, as well as preparing us for our entrepreneurial work for our future startup, with the produced biosensor.

Furthermore we would like to thank the DTU Health Tech team for their support and guidance throughout the project.

8 Appendix

8.1 Appendix to Stakeholder Desirability

As an addition to the stakeholder analysis in the Translational Potential section, the figure below illustrates how to communicate with the different types of stakeholders. As it can be seen, the most important is of course stakeholders that are high in both power and interest, and thus have incremental influence on the project and can make it both succeed as well as fail, if they are not kept in close contact and on-going dialogue about their needs.

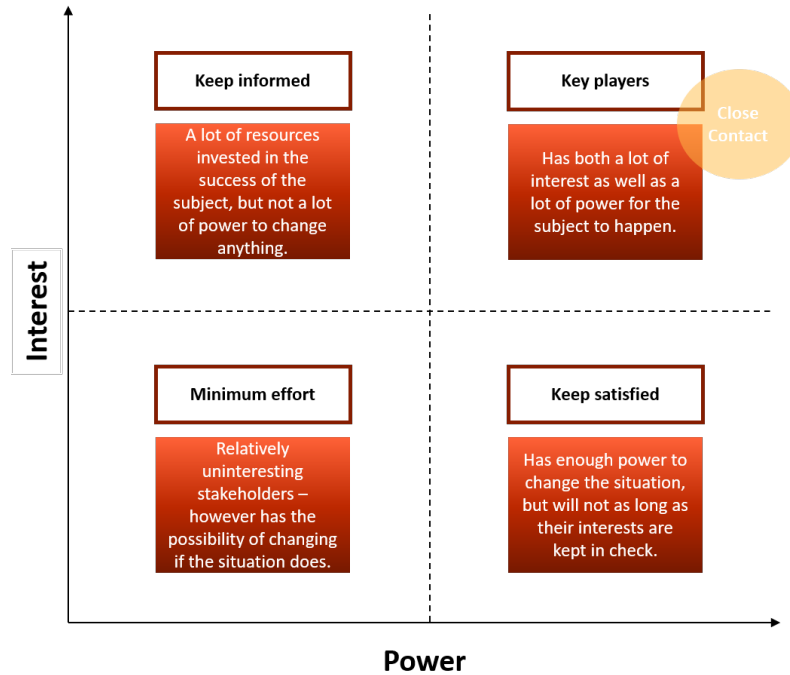


Figure 7: Explanation on how to handle the different stakeholders based on where they belong on the Power/Interest matrix in Figure 5.

8.2 Appendix to Business Feasibility

The market analysis has been based on the Porter's Five Forces model as illustrated on the figure below.

The figure indicates where the focus primarily lies in this initial phase of the start-up launching (Threat of Entry and Buyer Power), as well as what can be neglected for now (Supplier Power), as the suppliers are abundant and low in power, whereas the customer's power should be assessed more thoroughly through more close, direct communication. If the hospital administration can decide on the purchase themselves, the Buyer Power will be low, and the transaction will be a Business to Business sales, but if the government has to have a say, the power increases drastically - which will also create ripples in the stakeholder analysis.

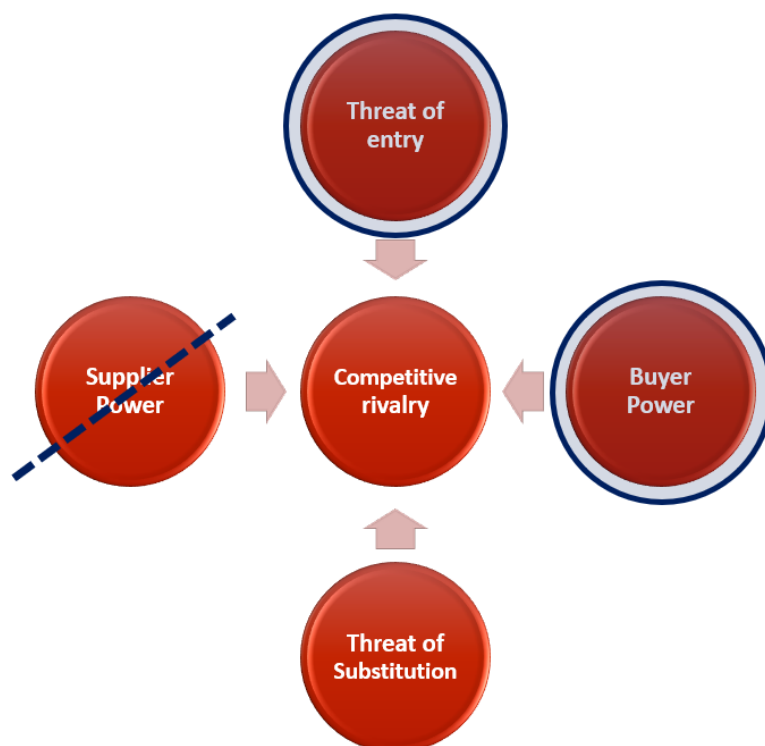


Figure 8: Illustration of Porter's Five Forces as a step in the preliminary market analysis.

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