CAREDIOBIT

YOUR BEATS

OUR BITS

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SUMMARY

Carediobit developed a real-time biosensor to detect NT-proBNP in plasma. The core of the device is a disposable glass fiber in which a light beam is guided through and reflected on a gold coated detection zone. Upon submerging the detection zone in the patient's sample, target molecules bind to the gold surface and cause a change in the reflected light. This change is monitored and translated into the target concentration.

The biosensing system is incorporated into a portable device which includes a specifically designed rotating system to easily perform the two-step assay. With minimal sample preparation (1:2 dilution), the sensor can detect clinically relevant concentrations in plasma within six minutes.

What makes Carediobit unique is that we, besides the developed test device, provide a full package of services in elderly care homes. This includes the necessary follow-up tests together with a treatment suggestion, comprising medication, dietary guidelines and a physical activity plan. In this way, Carediobit assists care takers in timely uncovering the first symptoms of heart failure and allows cost-effective periodic monitoring of the heart failure risk.

I. BIOSENSOR SYSTEM AND ASSAY

In order to detect the target molecule NT-proBNP, Carediobit uses a technique called Fiber Optic Surface Plasmon Resonance (FO-SPR) in combination with the sandwich ELISA principle. FO-SPR is a mass-based technique in which light is guided through a bifurcated optical fiber to a gold coated detection zone, causing surface plasmon resonance at the gold-sample interface. This resonance condition is characterized by a spectral dip at a certain wavelength of the incident light beam and depends on the refractive index of the sample. When the gold coated detection zone is functionalized with bioreceptors (e.g. antibodies (Ab)), specific binding of target molecules changes the refractive index of the sample and shifts the spectral dip to a different wavelength. This shift in wavelength can be related to a certain concentration of the target[1]. The main advantages of this technique are the real-time detection, label or label-free detection, small sample volume consumption and short time-to-result. As NT-proBNP is a very small molecule, signal amplification is required to obtain detectable wavelength shifts and hence, a sandwich-based assay is used. A sandwich ELISA realizes target detection by using a first Ab to capture the target and a second Ab to label the target (e.g. fluorescently). In our bioassay, capture Ab's were immobilized at the gold coated detection zone and labelling Ab's on gold nanoparticles (AuNP's). Once the target is captured, binding of the AuNP-coupled labelling Ab increases the mass of the target molecule, causing a bigger shift in wavelength and more sensitive detection of the target.

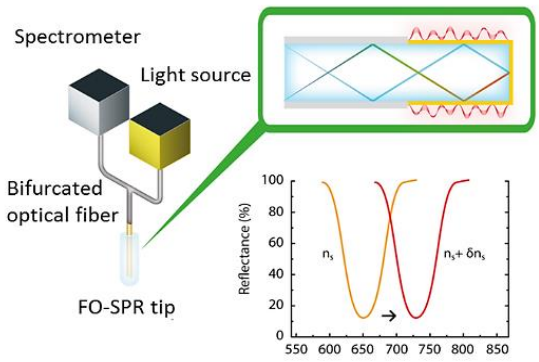

Figure 1. SPR principle

The following workflow guides through the assay. First, glass fibers are cut and covered with a thin gold layer. Next, a carboxylic acid terminated monolayer is applied on the gold surface and activated in order to attach the first layer of capture antibodies on the fiber. This step is done beforehand and the functionalized fibers are stored until further use. The NT-proBNP concentration of a patient is determined by submerging the functionalized fiber in a 1:2 diluted plasma sample. The target molecule binds to the capture antibodies (15C4, Hytest, Finland) previously conjugated onto the gold surface. Next, the fibers are submerged in a solution containing gold nanoparticles (ϕ 20 nm) pre-functionalized with detection antibody (15F11, Hytest, Finland) to amplify the signal. The amount of NT-proBNP that is bound, and thus was present in the sample, is determined by translating the shift in wavelength using a calibration curve.

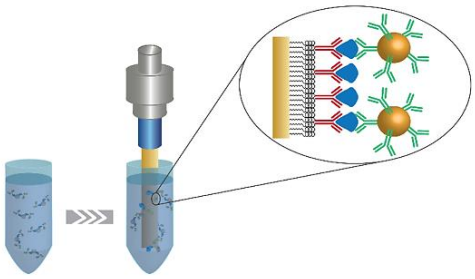

Figure 2. FO and Immunocomplex

Since the detection of biomolecules in the system is based on detecting changes in light characteristics, the first component of the biosensor system (Figure 3) is a light source, namely an LED (Luxeon Rebel, Lumileds, The Netherlands). The white light then travels through one side of a bifurcated fiber towards the functionalized fiber dipped in the sample. The shifted light travels back to the bifurcated fiber and to a micro-spectrometer (Hamamatsu, Japan). The spectrometer detects the wavelength and sends the values through a control board to a Raspberry Pi 3 computer (United Kingdom) every 15 ms. Using Python (United States) code which was programmed onto the Pi, the information is processed to calculate the slope of the data, as well as the difference with the calibration curve. After the Pi's calculations, the values are displayed on a small touch screen of Adafruit Industries (United States). The Pi and all the components attached to it, is powered by a battery and an expansion board. This board is needed for keeping the voltage constant and supplying the peripherals with the necessary current.

Figure 3. Carediobit's prototype

As at this point, the assay is a two-step process, an easy-to-use Eppendorf switching mechanism was developed to assure simple tube handling. A manual rotor was designed, equipped with a ball-spring system. This allows correct placement and locking of the sample vial with respect to the functionalized fiber, as well as the precise and quick change of vials during the test. The parts were produced by 3D printing, with the ball-spring system later inserted. Further development of the bioassay and measuring device will allow to perform one-step assays, thereby reducing the time-to-result as well as the complexity of the test and the device.

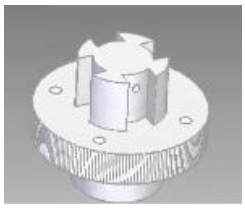

Figure 4. Switching mechanism

II. ANALYTICAL PERFORMANCE

Currently, measurements are performed in 200 µL of sample volume. The solution of gold nanoparticles has an optical density value of one. One measurement takes six minutes in total: target binding in 1:2 diluted plasma for five minutes and one minute gold nanoparticle binding for signal amplification. The slope of the second step is used to build the calibration curve in order to determine the NT-proBNP concentration in the sample.

The calibration curve (Figure 1) is based on measurements of samples with a known concentration (0, 100, 200, 400, 800, 1600, 3200 and 6400 pg/mL) and every concentration is repeated twice. All the measurements are conducted with the same principle: 100 µL of sample is diluted in 100 µL of PBS + 0.2%Tween 20. Since the sample is diluted twice, the final measured concentration is half of the original concentration in the sample. The error bars are the standard deviation based on two repetitions. Using the obtained curve, the NT-proBNP concentration (pg/mL) of an unknown sample can be calculated. The limit of detection was calculated based on three times the standard deviation of the blank and the NT-proBNP concentration was extrapolated from the calibration curve, reaching 420 pg/mL for the original sample.

In this and next week, the assay will be further optimized to allow measuring in a total volume of 40 µL, so the assigned contest volume of 20 µL sample will be diluted in 20 µL of PBS + 0.2% Tween 20.

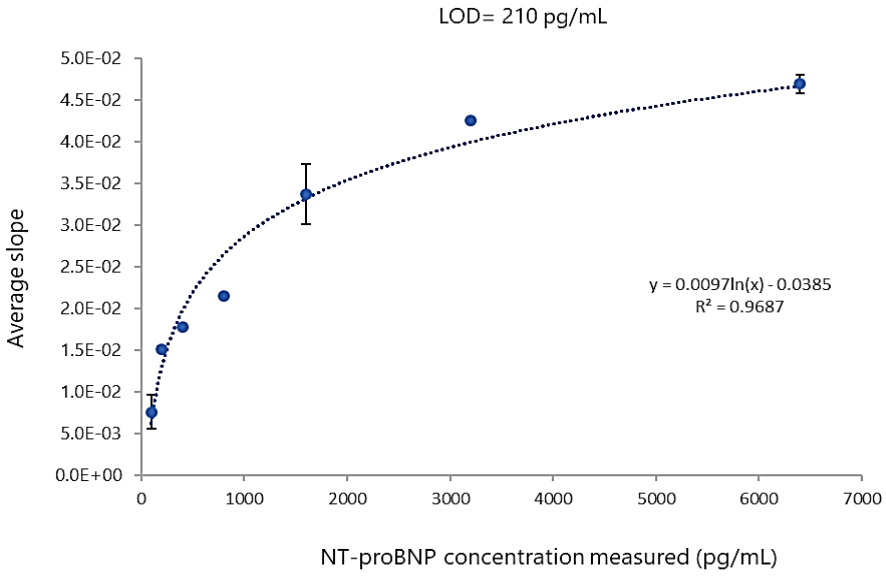

Figure 5. Calibration curve of two-fold dilutions of NT-proBNP in 1:2 diluted plasma ranging from 0 till 6400 pg/mL. A logaritmic trendline is plotted.

III. NOVELTY AND CREATIVITY

III A. ALREADY AVAILABLE

The concept of using FO-SPR for biomolecule detection is investigated by the MeBioS research group at KU Leuven. A benchtop FO-SPR device (FOx Diagnostics) was available for the initial antibody screening

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III B. NEW DEVELOPMENTS

The Carediobit team has developed a portable FO-SPR device from scratch and has shown the possibility of dry storage of antibody-functionalized fibers by incubating the functionalized fibers for 10 minutes in a 0.5% trehalose solution and drying in a contamination-free environment. So far, a good analytical performance after three days and one week of dry storage was shown, which is a crucial step for the commercialization of our device. Next, a novel antibody pair for the sandwich-based detection of NT-proBNP, giving high signal and low nonspecific binding in plasma samples, was selected. Besides, protocols for antibody functionalization and signal enhancement with gold nanoparticles were developed to meet the needs of our assay concept and target. Additionally, we designed an Eppendorf switching mechanism as the necessity of switching vials for the two-step test was a weakness regarding user friendliness and robustness of the test. We developed a manual rotor which allows vial switching in a predetermined and safe fashion and locks them in the correct position during the measurement. Alternatively, this can be upgraded to an automatic rotor to further reduce the handling steps of the device. Another very interesting feature is the use of a Raspberry Pi for the calculations. This microcontroller allows analyzing data normally performed on a computer, with the advantage of being much smaller. While taking advantage of the many available add-ons (e.g. display, buttons) but coding all the components ourselves, the interface could be fully adapted to the needs of our device.

IV. TRANSLATIONAL POTENTIAL

IV A. HEALTHCARE APPLICATION POTENTIAL

It is estimated that 1-2% of the total population of Europe suffers from heart failure, 80% of whom are 65 or older. In addition, this group of elderly will only be growing and is estimated to make up 29% of Europe's population by 2060[2]. This makes the elderly a high-risk group of patients[3] in the near future. Many of these people will decide to live in long-term residential care institutions (e.g. nursing homes or service flats) where they are provided with supervision and assistance in their everyday lives. In nursing homes, regulations require one nurse to be present per 30 patients or per facility floor, making it difficult to introduce personalized care. Moreover, the first symptoms of heart failure such as fatigue, weakness and dyspnea are largely neglected, since they are commonly attributed to old age. Diagnosis often comes too late and a manageable condition leads to death.

Policymakers stress that "we urgently need more uniform application of guidelines, equitable access to modern treatment, and holistic models of care"[4]. Today screening is not commonly done yet and patients are only referred to a cardiologist for an ECG when symptoms occur. However, early detection is crucial regarding the survival rates with heart failure and this is where Carediobit comes in. Our services assure cost-effective periodic monitoring with NT-proBNP along with an additional cardiac biomarker and a biomarker for Chronic Obstructive Pulmonary Disease, a condition with similar symptoms as heart failure but necessitating different treatment[5]. By introducing our services in elderly care homes, preventive screening and application of appropriate treatments can be implemented much more efficient. What makes us unique is that we would not only provide the diagnosis, but also the necessary treatment plan (comprising of medication, adjusted diets and physical activities) and follow-up tests. Moreover, based on the follow-up tests, treatments can be evaluated, adjusted and consulted. While frequent monitoring of diagnosed patients will save lives already, standardized screening will also prevent or postpone the onset in others and increase the overall life quality of elderly people

IV B. INDUSTRIALIZATION AND COMMERCIALIZATION POTENTIAL

FO-SPR is a robust technique proven to be suitable for the detection of different types of molecules (i.e. proteins, nucleic acids and small molecules) which may enable future testing of microvesicles or DNA markers, currently under investigation for their relevance in detecting and managing heart failure. Moreover, FO-SPR allows for direct readout in crude matrices such as blood without any purification needed[6]. Even compatibility with dry blood spots on filter paper has been proven to be enough for testing, being one of the greatest advantages assuring the efficiency of our system. Furthermore, innovative multiplex immunoassay concepts, towards an additional cardiac biomarker and a biomarker for Chronic Obstructive Pulmonary Disease, are explored together with the MeBioS Biosensorgroup of KU Leuven. FO-SPR is already approved for use as a research tool and secured by three patents, which makes the road to certification for diagnostic use shorter and allows a relatively quick entry to the market. Starting from our own benchtop research tool, we would initially provide our services at a centralized, specialized laboratory, collaborating with our partners FOx Diagnostics for the provision of technology (exclusive

licenses) and KU Leuven for innovative optimized assays. Carediobit would offer sample testing, diagnosis and follow-up tests at their facilities on the same day if the dried blood spot is sent before a certain time in the day. Initially, logistic services could be outsourced, while a secured online platform and patentable decision tree smartphone app would take care of reporting the results and communicating the necessary treatment plan (comprising of medication, adjusted diets and physical activities). The app would be introduced with our entry into the market. First, it would offer basic functions such as access to results and general dietary and physical activity advice. Gradually this online platform would be expanded, enabling caregivers to schedule appointments for more specialized tests. Finally, it would also show suggested tests and the assigned treatment for the patients. By 2019 the online platform and basic smartphone app service could be launched into Belgian nursing homes already counting > 150 000 residents. This market is expected to further increase by 2030 to > 190 000 and even > 300 000 by 2060[7].

The complete service mentioned above would be sold to the care facilities, with an annual fee for standard screening and personalized care plans, depending on the size of the facility and additional fees for more specialized tests. The tests and innovative treatments, as well as the proprietary decision tree for efficient diagnosis would be established in close collaboration with an interdisciplinary team of medical experts and a panel of Key Opinion Leaders. In contrast with certain private hospitals already offering personal treatment, our services would provide a fully integrated treatment plan, available for the general public. Starting its journey in Belgium, Carediobit could become a leading provider of fast and easy testing of blood droplets, focusing on nursery homes applications and aiming to extend their applications in a next stage.

Next, Carediobit will focus on (i) development of a point-of-care device, (ii) expansion towards the European market and (iii) generation of tests and treatments for other age-related diseases. Further collaboration with Comate could help achieving the goal of a more handheld device. This could then be leased to nursery homes with them paying for the tests. We envision that our miniaturized device would result into a smartphone-based system, only using a phone case to hold testing cartridges (with incorporated optical fibers) and the device's light source and camera for the readout. This would further increase the availability and sales market of our service. Next to elderly living in nursing facilities, a second big group of elderly still living at home and going to a GP can be reached. With this smartphone-based system, Carediobit wants to move from in-lab testing towards a point-of-care European market. Finally, investments would be made into clinical studies towards novel treatment and diagnostic combinations for other conditions related to old age.

V. TEAM AND SUPPORT

V A. CONTRIBUTIONS OF THE TEAM MEMBERS

Whereas the team members combined their efforts to reach the goal, they were channeled into three subgroups: the bioassay team, the transducer team and the signal processing team.

The bioassay team (Hanne, Pilar, Rodrigo, and Anke) made a well-substantiated decision on the combination of antibodies, optimized the bioassay and evaluated different ways of fiber storage. Rodrigo furthermore focused on writing a code for data analysis, Pilar on sponsor contacts, Anke on the website and Hanne on the sponsor brochure.

The Transducer team (Bram, Kinga, and Gaëtan) selected and combined the building blocks for our biosensor and designed the entire system. Kinga also focused on the translation and market strategy, Gaëtan contributed to the sponsor contacts and the website management and Bram was in charge of the 3D design of the several prototypes.

The signal processing (Shahin and Nina) installed and set up the Raspberry Pi and the touch screen and Nina also worked on the translation and market strategy.

V B. PEOPLE WHO HAVE GIVEN SUPPORT

We share the credit of our work with Professor Jeroen Lammertyn, Dr. Devin Daems, Dr. Deborah Decrop, Dr. Karen Leirs, Karen Ven and Bernd Peeters. It is with immense gratitude that Carediobit acknowledges the support and help of all the above mentioned.

Carediobit is also grateful to Hytest for providing NT-proBNP, antibodies and plasma and thereby contributing to our biosensor. Finally, Carediobit is thankful to SensUs to give us the opportunity of such a priceless experience, growing as scientists and human beings and bettering health care prospectives nowadays.

V C. SPONSORS

Carediobit considers it an honor to work with MeBioS, FOx Diagnostics, Future Diagnostics and Comate as our main sponsors. We thank them for their financial support and their advice, shared with us during many meetings.

Furthermore, we are thankful for the financial support of ADx NeuroSciences, LCIE, Melexis, KU Leuven and KU Leuven Research and development.

VI. FINAL REMARKS

In order to thank the sponsors for their financial and advisory support throughout the year, the Carediobit team organized a sponsor event. On this event, all sponsors were invited for an update on the team progress. The scientific progress and the business plan was presented and measurements were demonstrated on the final prototype, allowing the sponsors to share their final suggestions and remarks with the team. In addition, this event enabled the team members to get in touch with representatives from the sponsoring companies and expand their network with people form industry.

Moreover, to ensure the connection with the target audience and to contribute in yet another way, Carediobit voluntarily spend some time with the 'Dirk Verheydenfonds' charity, which is putting great effort in helping heart failure patients in Belgium. The charity is founded by Lucien and Lisette Verheyden, to honor their son who passed away because of heart failure. This charity focuses on gathering financial support for research at the cardiac research Fund Aalst. We met to exchange our stories, learn from them and talk about the potential impact of our biosensor. They also explained how they cooperate with Fund Aalst, how they keep track of the latest achievements in the field and actively organize fundraising activities at a local level. Although Carediobit and the Dirk Verheydenfonds use different approaches to tackle the world spread issue heart failure is becoming nowadays, we feel connected in our contributions towards a better understanding of the disease and to prompt heart failure diagnosis and treatment in an easy, non-invasive and economic manner.

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