LABEL-FREE AND PROTEIN G-ENHANCED OPTICAL FIBER BIOSENSOR FOR DETECTION OF ALDH1A1 CANCER BIOMARKER

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT OF NAZARBAYEV UNIVERSITY FOR THE DEGREE OF MASTER OF SCIENCE IN BIOLOGICAL SCIENCES AND TECHNOLOGIES

APRIL, 2025

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Abstract

Cancer remains one of the leading causes of morbidity and mortality worldwide, highlighting the need for improved early detection strategies. Aldehyde dehydrogenase 1A1 (ALDH1A1) has emerged as a significant biomarker associated with tumor progression, chemoresistance, and poor prognosis in various cancers, including breast, lung, colorectal, prostate, and lymphoma. Current diagnostic methods for ALDH1A1, such as flow cytometry and ELISA, are limited by long detection times, the need for labeling, and reduced sensitivity in complex biological matrices.

This study presents a novel optical fiber biosensor based on magnesium silicate nanoparticle-doped fibers for the label-free detection of ALDH1A1. The biosensor design incorporated protein G for enhanced antibody orientation and binding efficiency and anti-ALDH1A1 antibodies for specific recognition. Several sensor configurations were fabricated using a semi-distributed interferometer (SDI) format, and their performance was evaluated across a wide concentration range (10 fM–100 nM) in both phosphate-buffered saline (PBS) and fetal bovine serum (FBS).

Our findings demonstrated that the inclusion of protein G significantly improved sensor sensitivity and reproducibility, achieving a limit of detection (LoD) of 172 fM in PBS. The sensor also maintained a positive response trend in FBS, indicating its potential applicability in clinically relevant samples. Furthermore, specificity testing confirmed the sensor's selectivity toward ALDH1A1, with negligible response to vascular endothelial growth factor (VEGF). This work introduces the first reported optical fiber biosensor for ALDH1A1 detection, offering a rapid, label-free, and highly sensitive approach suitable for future development in cancer diagnostics.

Label-free and protein G-enhanced optical fiber biosensor for detection of ALDH1A1 cancer biomarker

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Abbreviations

ALDH1A1	Aldehyde Dehydrogenase 1 A1
AFM	Atomic Force Microscopy
CRC	Colorectal cancer
dB	Decibel
EBF	Enhanced backscattering fiber
EM	Electron Microscopy
FBS	Fetal Bovine Serum
sHER2	Soluble human epidermal growth factor receptor 2
MIP	Molecularly imprinted polymers
NHL	Non-Hodgkin's lymphoma
SDI	Semi-distributed interferometer
SMF	Single-mode fiber
PBS	Phosphate-buffered saline
RIU	Refractive index unit
ROS	Reactive oxygen species

Acknowledgments

First and foremost, I would like to express my deepest gratitude to my supervisors, Dr. Daniele Tosi and Dr. Timo Burster, for their invaluable guidance, continuous support, and encouragement throughout my research journey. Their expertise and mentorship have been instrumental in shaping this work. A special note of thanks goes to Dr. Aliya Bekmuzayeva, who has played a crucial role as a mentor. Her insight, patience, and constant support have greatly enriched my academic experience.

I am also sincerely thankful to all my lab members for their help and advice, particularly Maham Fatima, Marzhan, Kuanysh, Kanagat, Sabira, Toheeb, Aidana, Alina, and Damir. Their assistance, both in and outside the lab, has made this journey more enjoyable and productive.

Lastly, I am deeply grateful to my family and my friends, both from the biology department and other disciplines, for their unwavering support, encouragement, and belief in me through every step of this process.

Declaration

I declare that the research contained in this thesis, unless otherwise formally indicated within the text, is my original work. The thesis has been written by me in its entirety. I duly acknowledged all sources of information that have been used in the thesis. The thesis has not been previously submitted to this or any other university for a degree and does not incorporate any material already submitted for a degree.

Signature:  Date: 20.05.2025

1. Introduction

1.1. Cancer and ALDH1A1

Cancer is a multifactorial disease characterized by uncontrolled cellular proliferation, genetic mutations, and the ability to invade surrounding tissues and metastasize to distant organs. Despite advances in treatment modalities, the global burden of cancer continues to rise, with over 19 million new cases and nearly 10 million deaths reported in 2020 alone (Sung et al., 2021). Among top cancer cases, there are lung, female breast, colorectal, and prostate cancers in 2022, with 12.4%, 11.6%, 9.6%, and 7.3% of all cases, respectively (Bray et al., 2024). Early detection remains a critical factor in improving survival rates and treatment efficacy. In this context, early detection of malignant cancer development in patients is crucial, and biomarkers come into play as a crucial tool. Biomarkers are biological molecules (i.e., proteins, nucleic acids) that are measurable and serve as indicators of normal biological processes or pathogenic processes or responses to therapeutic interventions (Strimbu & Tavel, 2010). In cancer diagnostics, they can be found in tissues, blood, urine, or other body fluids. Therefore, biomarkers serve several important functions in cancer research and the medical field to help patients, such as early detection, screening, staging, and monitoring of cancer disease progression.

One of these biomarkers that has been of huge interest among researchers is aldehyde dehydrogenase. Aldehyde dehydrogenase (ALDH) is a superfamily of enzymes consisting of 19 isozymes. In the cell, they are responsible for processes like inhibition of reactive oxygen species (ROS) production, detoxification of reactive aldehydes into carboxylic acids, and synthesis of retinoic acid (Poturnajova et al., 2021; Xia et al., 2023). Especially the ALDH1A1 and ALDH1A3 isoforms, because they showed drug and radiation resistance. Specifically, ALDH1A1 was upregulated in breast cancer and lung cancer and reported as the reason for chemoresistance to cyclophosphamide (Sladek et al., 2002), doxorubicin (Croker & Allan, 2011), and paclitaxel (Sun et al., 2011), respectively. Moreover, increased expression of ALDH1A1 was reported in specific types of cancer diseases. For example, among 424 patients diagnosed with colorectal cancer (CRC), ALDH1A1 was elevated in tissues from 75% and 65% of patients either presenting lymph node metastasis (Yang et al., 2018). In malignant prostate cancer cells, there was a positive correlation between the prostate cancer growth, malignancy, and ALDH1A1 presence (Li et al., 2009). In the case of non-Hodgkin's lymphoma (NHL), there was a strong correlation between high expression of ALDH1A1 and poor prognosis in NHL (Song et al., 2018).

1.2. Diagnostics of ALDH1A1

Recent advancements in molecular biology and high-throughput technologies, including next-generation sequencing and proteomics, have accelerated the discovery and validation of novel cancer biomarkers. Despite this progress, challenges in translating these biomarkers into routine clinical practice still exist. Issues such as sensitivity, specificity, reproducibility, and accessibility need to be addressed to maximize their impact. There is ALDEFLUOR, which is an agent used in flow cytometry that emits green fluorescent light in ALDH-expressing cells (*ALDEFLUORTM Kit for ALDH Assays*, n.d.). Despite ALDEFLUOR being reliable and efficient, it bears slightly low contrast due to its "always-on" feature and is used *in vitro* only. There is an alternative called CS5-A and CS7-A, which works in deep red or near-infrared spectra and turns on only after interaction with ALDH1A1 (Oe et al., 2021). Another popular tool for ALDH1A1 detection is the ELISA kit with the sensitivity of 220 pg/mL (*Human ALDH1A1 ELISA Kit, Colorimetric, 90-min ELISA (Ab214024) | Abcam*, 2022), and it was used in the detection of the protein in non-small cell lung cancer (NSCLC) taken from patients (Rossi et al., 2019). Despite those methods being sensitive to ALDH1A1 and being utilized reliably, even for *in vivo* applications sometimes, they rely on labeled fluorescent molecules or suffer from background noise. Most importantly, the detection time takes from 15 minutes to 90 minutes.

Optical fiber biosensors are another alternative for developing a sensing tool that is not only as reliable and sensitive as the abovementioned tools but also fast. And some variations can be made label-free. The binding of target molecules on the tip of the fiber changes the incident light's intensity or wavelength, and the light is reflected back to be recorded by the interrogator. There are many types of optical fiber biosensors, depending on the method of fabrication and sensing material. Optical fiber biosensors offer advantages like high sensitivity and specificity, real-time and label-free detection, miniaturization and portability, immunity to electromagnetic interference, and working with complex samples (blood, serum, saliva) (Bekmurzayeva et al., 2022; Sypabekova et al., 2022). They can be metal-based, nanoparticle-based, and attached with receptors like antibodies, aptamers, or molecularly imprinted polymers (MIP) (Lyu, 2022). Successful utilization of optical fiber biosensors like ball tip fibers for detection of colon and gastric or breast cancers was reported earlier for the CD44 biomarker (Bekmurzayeva et al., 2022) and sHER2 (Sypabekova et al., 2022), respectively, at ultra-low-level concentrations. Recently,

Mg-silicate-based nanoparticle-doped optical fibers have been used to detect vascular endothelial growth factor down to the 26.6 fg/mL level for the purpose of fabricating biosensing tools for early diagnosis of diabetic retinopathy (Seipetdenova et al., 2025).

As of now, there are no reported optical fiber biosensors designed to detect ALDH1A1 proteins, especially label-free ones. Specifically, surface plasmon spectroscopy, interferometric biosensors, optical fiber biosensors, surface-enhanced Raman scattering (SERS) biosensors based biosensors for the detection of ALDH1A1 have no established data currently. Only fluorescent probes targeting cytosolic ALDH1A1 are present as discussed in sections above. Therefore, this project aims to cover this gap in knowledge and explore the possibility of designing optical fiber biosensors for the detection of the ALDH1A1 biomarker. However, it is important to find out normal level of presence of the ALDH1A1 in the blood because ultimate goal of this research is to develop a biosensing tool that can detect the protein the human serum. Serum analysis in healthy individual and patients with the lung cancer have shown that normal ALDH1A1 levels range between 5-8 ng/mL in healthy controls, while protein levels above 10 ng/mL was considered to be found in patients with the lung cancer (Rossi et al., 2019). Another study on serum ALDH1A1 levels based on chemiluminescence sandwich immunoassay indicated that normal levels of the protein ranges around 1.05 ng/mL and it depends on the kit sensitivity (Kong et al., 2018).

1.3. Protein-G and optical biosensors

Ideas of enhancing the sensitivity and specificity of biosensors were of great interest from the early years of biosensor development. One of the targeted ways in this field is utilization of protein G as a scaffold to increase the functionalization success and make the orientation of antibodies used for detection specific and better, thus increasing the overall sensitivity of the biosensor (Ebrahim-Habibi et al., 2019). Gold nanorods and gold nanoparticles treated with protein G prior to antibody immobilization have shown increased sensitivity; a gold nanoparticle-based sensor even showed ultra-low detection of 0.34 pg/mL (Centi et al., 2018; Elshafey et al., 2013).

1.5. Aims & Hypothesis

In this project, Mg-silicate nanoparticles-doped optical fiber in combination with protein G will be used to fabricate an interferometer functionalized with anti-ALDH1A1 antibody for the detection of the ALDH1A1 biomarker.

The hypothesis for this project is that an anti-ALDH1A1-based optical fiber biosensor will be able to successfully detect ALDH1A1 proteins in PBS and FBS. The modification with protein G before antibody immobilization should increase sensitivity.

To test the hypothesis, the following aims were set:

1. Fabricate SDI sensors with different modifications (with antibody only, with protein G and antibody, etc.).
2. Detect ALDH1A1 proteins of various concentrations in PBS and FBS.
3. Check for the specificity of the sensor.

2. Materials and Methods

2.1. Materials

HYPERION si255 optical backscatter reflectometer, shaker, standard single-mode fiber (SMF-28), enhanced backscattering fiber (EBF) made of Mg-silicate nanoparticles, standard telecom splicer (Fujikura 36-S), fiber cleaver (Fujikura CT-08), sucrose solution, phosphate-buffered saline (PBS), Piranha solution, methanol, 3-aminopropyl trimethoxysilane (APTMS), oven, glutaraldehyde, protein-G, anti-ALDH1A1 antibody, methoxy polyethylene glycol (mPEG), ALDH1A1 protein (target biomarker).

2.2. Methods

2.2.1 Fabrication and calibration of SDI fibers

Standard single-mode fiber (SMF) and enhanced backscattering fiber doped with nanoparticles are used in a simple fabrication process named “splice and cut,” which results in a semi-distributed interferometer (SDI). Basically, SMF and EBF are fused together, creating one long fiber structure. Then, joint EBF is cut in a way that approximately 1 mm of length remains connected to the SMF, which results in a cavity. The illustration of the process is shown in Figure 1. The cavity plays the role of a mirror that reflects back some of the incident light coming from the interrogation system.

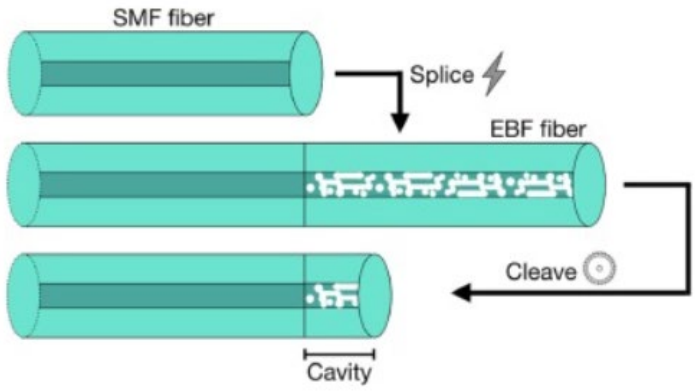

Figure 1. Fabrication and interrogation of the SDI interferometer for RI sensing, highlighting the splice-and-cleave operation. Adapted from *"Semi-distributed interferometers fiber-optic sensors for high-sensitivity refractive index detection: Design and sensitivity analysis,"* by S. Kazhiyev et al., *Measurement*, *220*, 113327 (2023).

Fabricated SDIs are further calibrated by using sucrose solution. Fibers are put into a custom-made plastic vial with 6 mL of 10% sucrose solution, and the signal reflected back by the EBF side is recorded by the HYPERION si255 optical backscatter reflectometer (Figure 2(d)). In a stepwise manner, 400 uL of 40% sucrose solution is added 5 more times, and the signal is recorded after each addition. The setup for the calibration experiment is shown in Figure 2(e). This gradual increase in concentration creates a gradient of refractive index change, which affects the reflected light signal and is recorded via the instrument. Later, this data is analyzed using MATLAB software, and the sensitivity values of fibers are obtained. The fibers are filtered out by their sensitivity: the fibers with good sensitivity are considered to be ones with maximum sensitivity values above 50 dB/RIU.

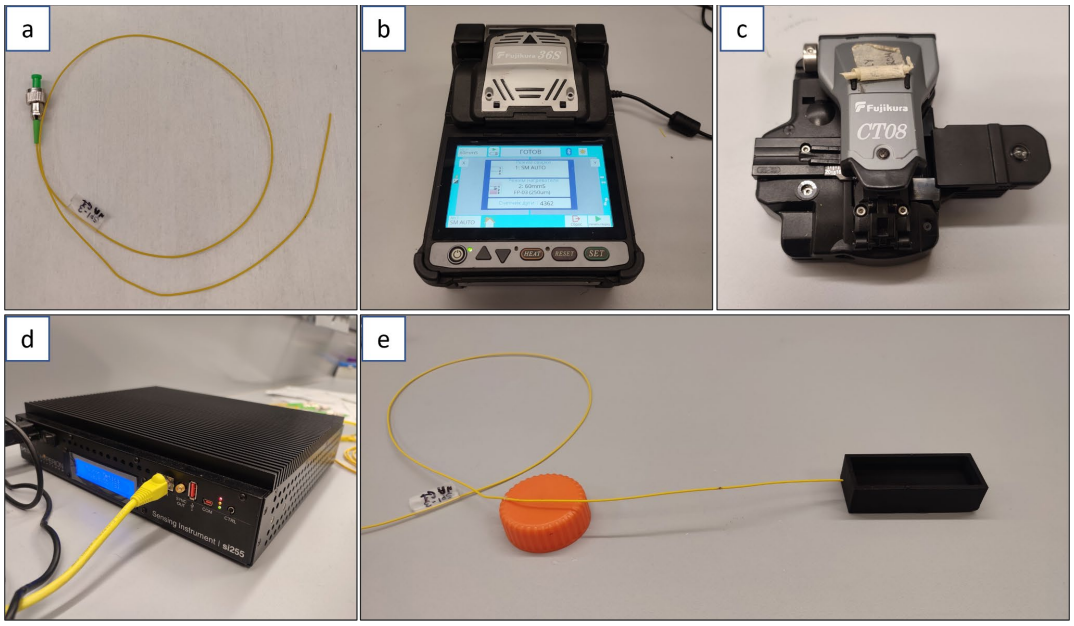

Figure 2. A collage of lab tools and instruments used for experiments. (a) Standard SMF-28. (b) Fujikura 36-S splicer. (c) Fujikura CT-08 cleaver. (d) HYPERION si255 optical backscatter reflectometer. (e) Setup for calibration experiment based on measurement of various sucrose concentrations.

2.2.2 Functionalization of SDI fibers with antibodies

First, Piranha solution (sulfuric acid + hydrogen peroxide in a 4:1 ratio) is used for 15 minutes to clean any organic residues and increase the hydroxyl groups (-OH) on the surface of the tip. After treating with the Piranha solution, fibers are rinsed with distilled water and left to air dry, or they are dried with nitrogen gas. Later, fibers are immersed in a 1% APTMS in methanol solution for 30 minutes for the silanization process. As a result, the surface of the fiber tip is modified to have amino groups attached to it. Next, fibers are heated in the oven at 110°C for 1 hour to facilitate the cross-linking between silane molecules. Then, fibers are incubated for another hour in 25% glutaraldehyde (GA) in PBS solution. Silane groups attached in the previous step enable binding of GA molecules, modifying the fiber tip further. This step is crucial because GA is susceptible to further modifications, and it can create a cross-link between proteins (i.e., antibodies) and other organic molecules.

After chemical modification, we step into the phase of immobilization of antibodies on the surface of the fiber tip. Due to modifications done in previous steps, antibody molecules are able to attach to the surface of the fiber tip. For this, fibers are inserted into the PBS solution with 4 ug/mL of antibodies and incubated for 1 hour. In case of modification with protein-G, first, fibers

are incubated in protein-G (1 ug/mL) solution in PBS for 1 hour, and then, they are incubated in antibody solution. After these steps, fibers are put into a 10% mPEG solution in PBS for blocking purposes. Free spaces of GA cross-linkers are blocked by mPEG molecules to decrease the chances of random binding of proteins during the detection. Some fibers are not immobilized with antibodies but treated with mPEG blocking solution to use them as a negative control.

The overall schematic of functionalization is shown in Figure 3 below.

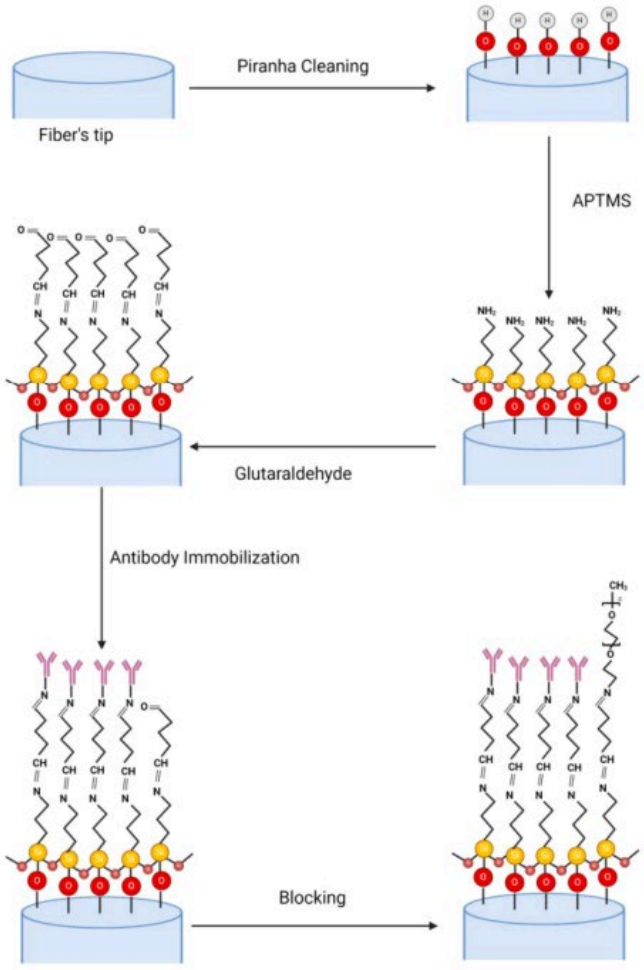

Figure 3. A schematic of the functionalization process of the fiber tip surface. Adapted from *"Label-free multiplexed detection of diabetic retinopathy biomarkers using fiber optic biosensors: Towards lab-in-the-tear,"* by S. Seipetdenova et al., *Optics and Lasers in Engineering, 189*, 108943 (2025).

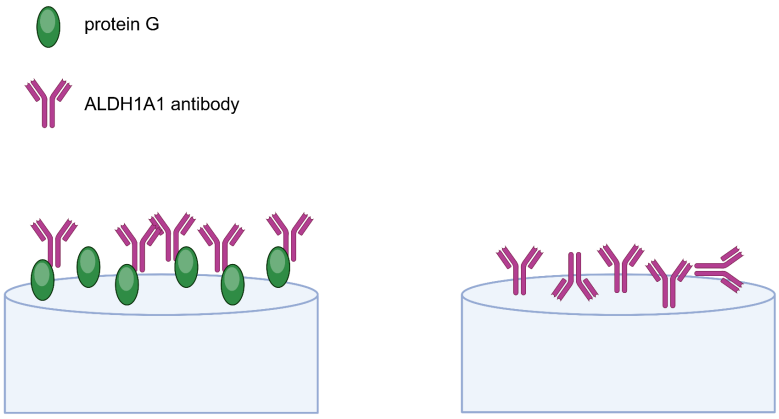

Figure 4. The illustration of oriented immobilization of ALDH1A1 antibody by protein G on the fiber tip surface.

Based on this theory, successful orientation of antibodies on the surface of the tip is expected, thus increasing the sensitivity of the fiber biosensor during the detection of the biomarker.

2.2.3 Detection of biomarkers

Successfully functionalized fibers are further used in the detection. ALDH1A1 protein solutions with various concentrations are prepared and used for the detection purposes. The ALDH1A1 protein biomarker is diluted in the PBS. Starting concentration is considered to be PBS (0 nM ALDH1A1). The lowest ALDH1A1 protein concentration used is 10 fM and the highest concentration is 100 nM, respectively. Fibers connected to the HYPERION si255 instrument are placed into the plastic cab with 200 uL of either PBS or protein solutions. Biomarkers are measured starting from the lowest to the highest concentration. Each concentration of biomarkers is measured for 10 consecutive minutes, and the signal is detected every minute. Obtained data is analyzed via MATLAB software, and the detection curves are designed. The illustration of the experimental setup is shown in Figure 5 below.

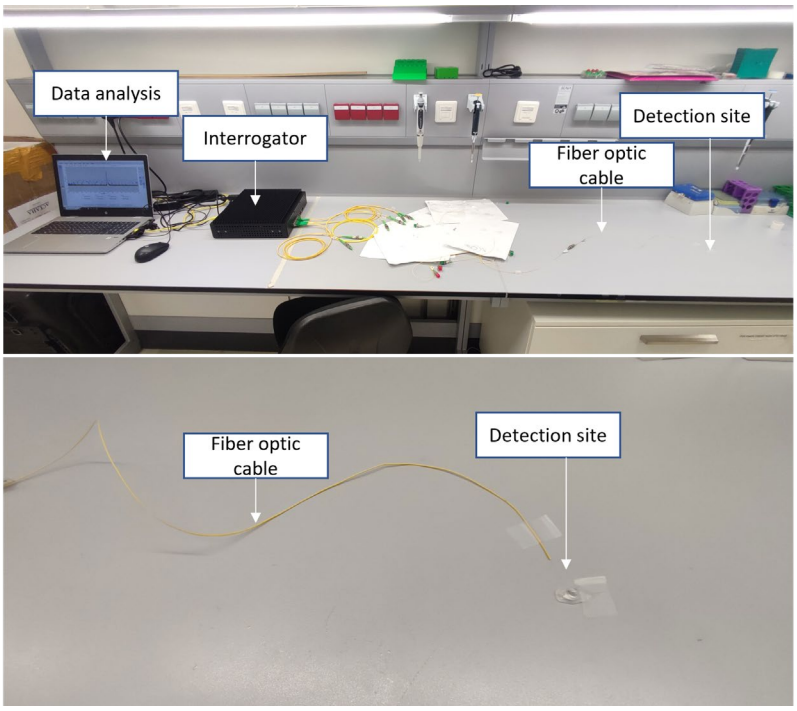

Figure 5. A photo of an experimental setup designed for detection of the ALDH1A1 biomarker.

3. Results

3.1 Calibration analysis

After SMF-28 fibers were spliced with nanoparticles, they were filtered out based on a calibration experiment. Fibers that showed sensitivity of >=50 dB/RIU or over were selected for further modification. In the figure below on the left, the signal spectra of the SDI fiber at various wavelengths in sucrose solution is shown. The highest peak in the spectra belongs to the FBG fiber, which plays the role of a reference. In the right picture, the sensitivity spectra of the same SDI fiber with its peaks and valleys is illustrated. This specific SDI had a maximum sensitivity of 92.4 dB/RIU, but that was not the only factor to be considered. Fibers sensitivity spectra were also analyzed for overall peaks and valleys. In general, this SDI had several valleys with good sensitivities closer to the maximum sensitivity. Based on these two factors, fibers were used in the next functionalization step.

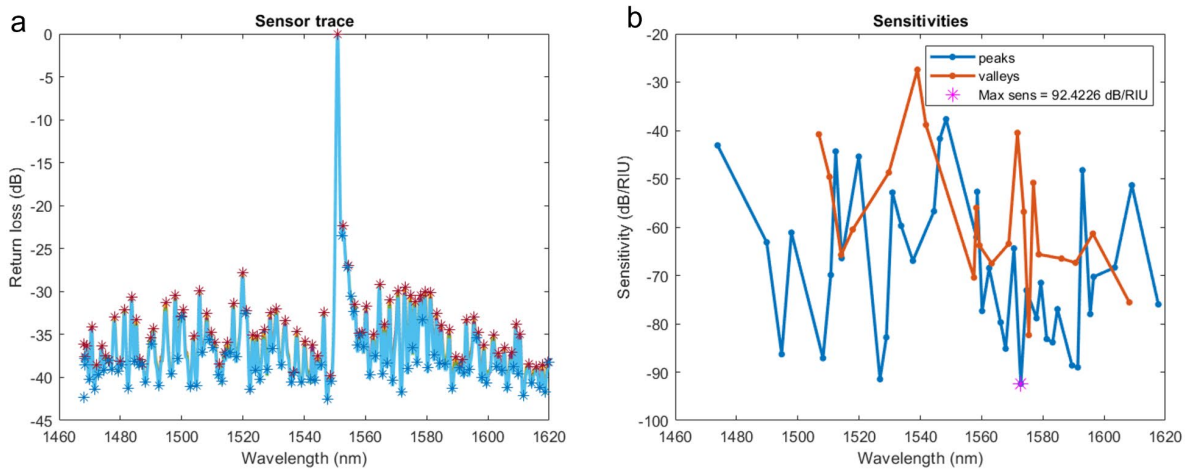

Figure 6. (a) Spectra of the SDI sensor in sucrose solution. (b) Sensitivity spectra of the SDI sensor. Maximum sensitivity at 1572 nm is 92.4 dB/RIU.

3.2 Biomarker detection analysis

Based on initial analysis, general trends for change in the signal intensity in peaks and valleys were figured out. Here, in Figures 7 and 8, signal intensity changes of two differently functionalized SDIs are shown. Figure 7 belongs to the SDI fiber that was functionalized by skipping the antibody immobilization step. Subsequently, this SDI didn't have any antibodies on the surface of the tip, and it showed the expected outcome during the detection of the ALDH1A1 biomarker. As a result, on almost all peaks and valleys, there is no or insignificantly little change in the signal intensity. However, SDI that was treated with protein-G and then with antibodies (Figure 8) had a general trend of signal reduction throughout various ALDH1A1 concentrations from 10 fM to 100 nM. These and other similar data, depending on the treatment conditions, were further analyzed to plot the slope for sensitivity.

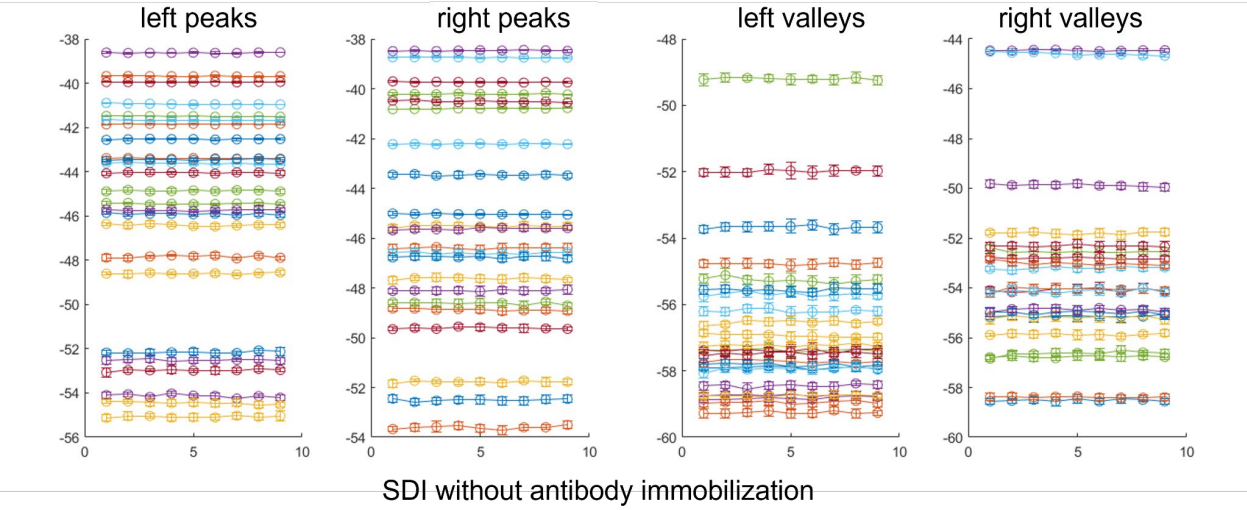

Figure 7. Signal intensity of the SDI sensor without immobilized antibody after the detection of ALDH1A1 in PBS.

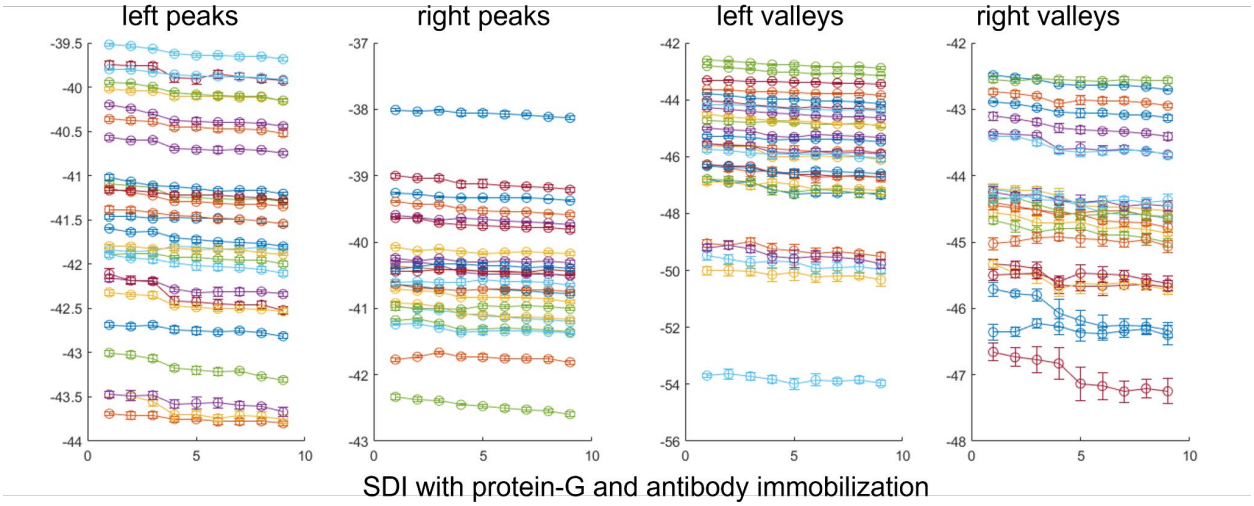

Figure 8. Signal intensity of the SDI sensor treated with protein G and immobilized antibody after the detection of ALDH1A1 in PBS.

The heatmap of one of the SDI sensors treated with protein-G and antibodies is shown below. It is another way of displaying the change in the signal intensity. The upper axis shows the scale for the dB change in the signal response, while the lower axis indicates the ALDH1A1 concentrations starting from 10 fM to 100 nM. Also, there is a timescale that is introduced on the lower axis, which shows that each concentration was measured for 10 consecutive minutes. The left axis shows the wavelengths of light that were tracked back by the interrogator.

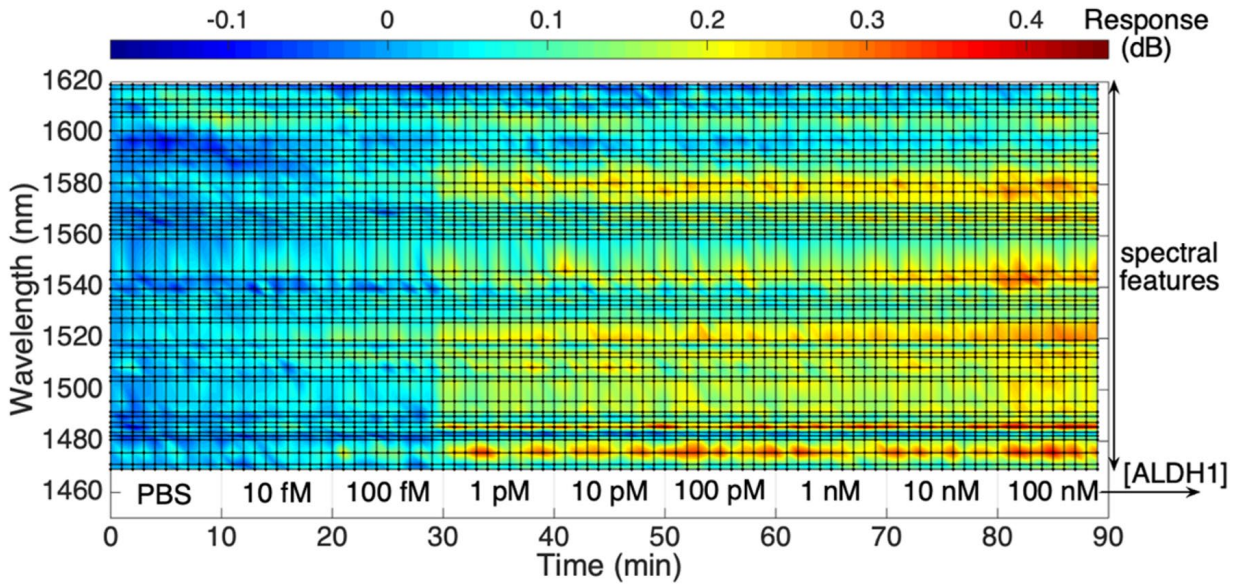

Figure 9. Heatmap of one of the representative SDI sensors with spectral peak response regarding the concentration of ALDH1A1 protein in PBS from 10 fM to 100 nM.

3.3 Overall comparison of different SDI biosensors and measurement conditions

Figure 10(a) displays slopes of signal response change of different SDI sensors after the detection of various concentrations of ALDH1A1 (10 fM - 100 nM). Slopes were made based on mean values of repetitive experiments. Error bars indicate the standard deviation from mean values. As we can observe, SDI fibers with or without protein-G treatment and without immobilized antibodies had a straight slope with the lowest values, which indicates they were not sensitive to the protein's concentration change. On the other hand, SDIs immobilized with antibodies showed a positive slope several times larger than previous groups discussed. SDIs not treated with protein-G and treated with 1 ug/mL protein-G before antibody immobilization demonstrated an increase in response (dB/concentration) and had positive slopes such as 0.0127 dB and 0.0178 dB, respectively. Also, it is worth noting that SDIs treated both with protein-G and antibodies (4 ug/mL) had a higher slope compared to the group only treated with antibodies. Moreover, standard deviation is much smaller in the last group, which indicates the enhancement of sensitivity due to protein-G treatment. In addition, further analysis of the last group revealed to us the limit of detection (LoD) for this kind of biosensor, which was equal to 172 fM, as shown in Figure 10(b). As the results based on the signal change (in dB), the PBS (conc – 0 ug/mL) signal

was set as a background and removed from the signal peaks' value (in dB) of other concentrations (10 fM – 100 nM). Then, that difference is graphed on figures below.

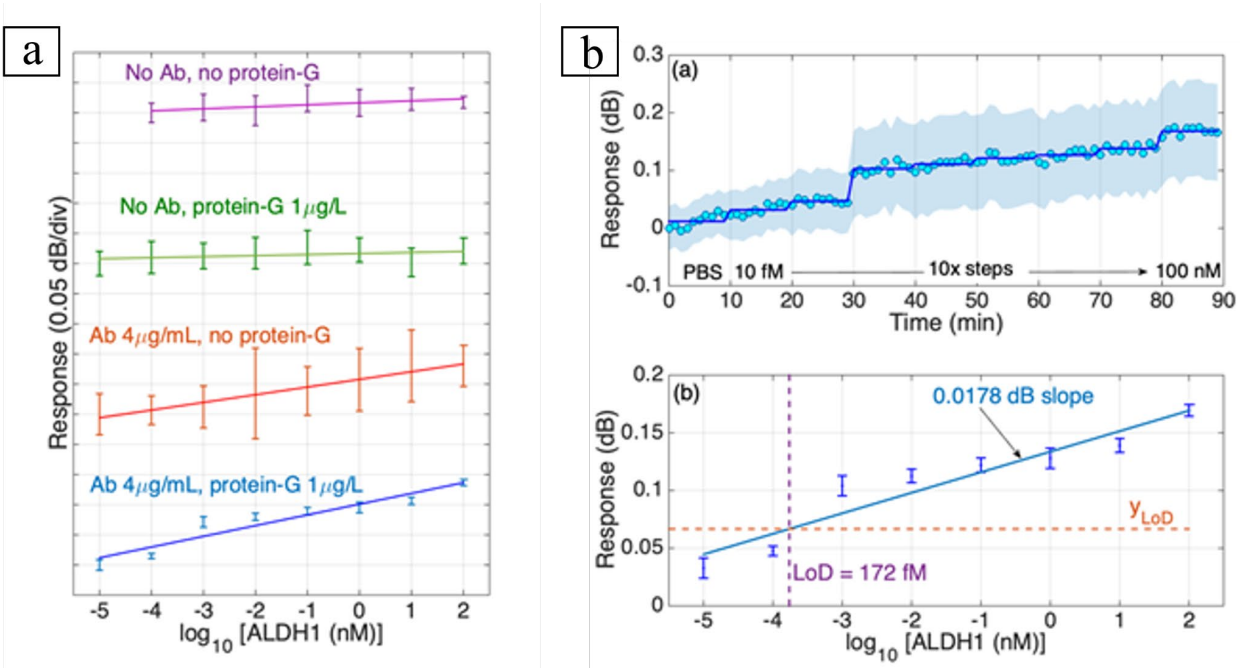

Figure 10. Response of anti-ALDH1A1 antibody immobilized SDI sensors during the detection of the ALDH1A1 biomarker in PBS. (a) Comparison of response of SDI sensors functionalized differently in detection of ALDH1A1 protein with various concentrations from 10 fM to 100 nM in PBS. Slopes for each condition (from top to bottom): 0.0033 dB, 0.0017 dB, 0.0127 dB, and 0.0178 dB. (b) Mean response of SDI sensors treated with protein-G and immobilized with anti-ALDH1A1 antibody in detection of the biomarker (ALDH1A1).

Later, biomarker detection experiments in FBS, which is closer in complexity to human serum compared to PBS, also showed a positive correlation between an increase in the ALDH1A1 concentration and the response signal. The SDI modification was the same as in the most successful 4th group. Fibers were treated with both protein G and antibodies. Despite the slope of the sensitivity being smaller than the group tested in PBS, it was still higher than the group with only antibodies (Figure 11).

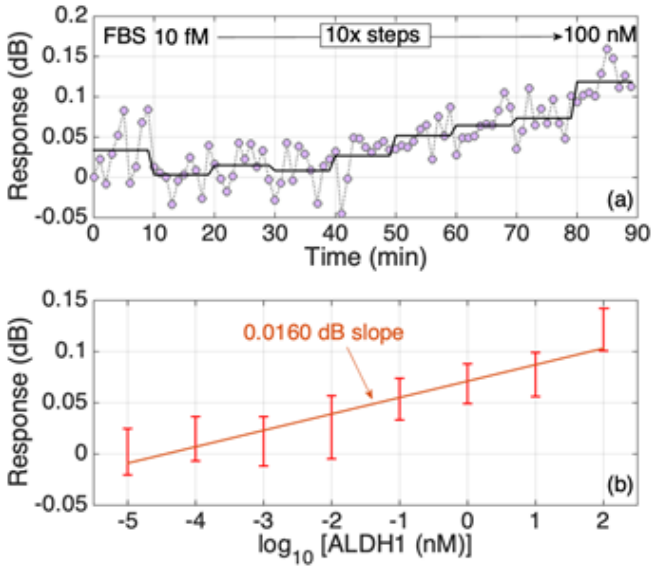

Figure 11. Response of anti-ALDH1A1 antibody immobilized SDI sensors during the detection of ALDH1A1 biomarker in FBS from 10 fM to 100 nM.

To test SDIs with immobilized anti-ALDH1A1 antibodies, fibers treated with 1 ug/mL protein-G and 4 ug/mL antibodies were tested to detect VEGF protein from 10 fM to 100 nM concentrations in PBS. As Figure 12 shows, the slope is very small, indicating that there was no or very little sensitivity regarding the increasing concentration of VEGF. The slope of this group is closer to the slope of the SDI group not treated with antibodies.

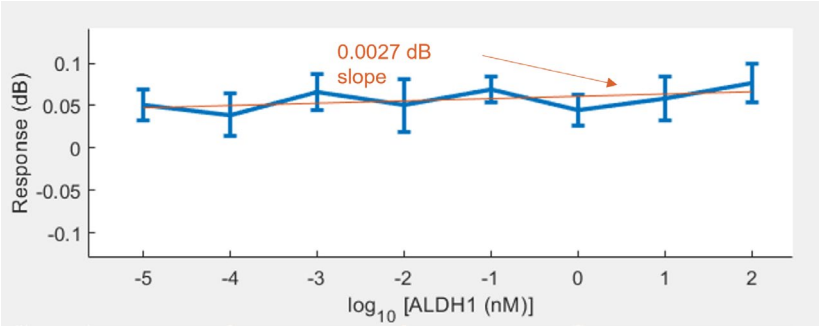

Figure 12. Response of anti-ALDH1A1 antibody immobilized SDI sensors during the detection of VEGF biomarker in PBS from 10 fM to 100 nM.

4. Discussion

This project successfully showed the efficacy of a novel approach of combining existing methods to increase the sensitivity and specificity of SDI fibers with anti-ALDH1A1 antibodies to detect ALDH1A1 biomarkers. The experiments demonstrated a reliable fabrication and functionalization process of SDIs for the detection of the ALDH1A1 biomarker at femtomolar levels. Moreover, protein G treatment of fibers before antibody immobilization proves to be efficient in increasing the sensitivity of the fiber. Despite that there is still a work to be done in this project. Based on the ALDH1A1 size, we can figure out what is the molar LoD for this project. The mass of the ALDH1A1 is 55kDA, so based on that we find out that 172 fM is 9.46 ng/mL. Sensitivity is higher than the normal ALDH1A1 levels of healthy patients, but capable to detect the lung cancer patients ALDH1A1 in blood as it had been shown to be higher than 10 ng/mL (Rossi et al., 2019).

However, we can observe a slight decrease in the signal response slope throughout the ALDH1A1 concentrations of the ALDH1A1 tested in FBS. Even the standard deviation became larger compared to the groups tested in PBS, despite being functionalized the same way. Probably, the presence of many different proteins and other biological substances creates some random binding or background noise. So, it may indicate that as the detection media becomes complex, it affects the sensitivity even on a smaller scale. An important thing to note is that anti-ALDH1A1 antibodies used in experiments were collected from ascites of the mice. So, some remnants of not filtered ascites might have played a role in random binding of other proteins during the measurement in FBS.

Still, there is a lot to adjust to optimize the protein G and antibody concentrations for even more effective detection of targeted proteins. This is definitely a beginning of new explorations and enhancements towards the goal of manufacturing fast, label-free, and highly sensitive biosensors.

5. Limitations

While this study demonstrated promising results in the development and application of SDI fiber biosensors for the detection of the ALDH1A1 biomarker, several limitations must be acknowledged:

Although the sensors were tested in PBS and FBS, which served as controlled and semi-complex media, they were not validated in actual human serum or other clinically relevant biological fluids yet. The presence of real samples, such as proteins, lipids, and salts, may affect specificity and sensitivity due to non-specific binding or signal noise.

The current experimental design allows for the detection of a single biomarker, which is ALDH1A1. For practical applications, a multiplexed platform, detection of several biomarkers, would be more effective and specific to the cancer type.

Also, topology studies either with electron microscopy (EM) or atomic force microscopy (AFM) have not been addressed in this study.

6. Summary and future perspectives

To move forward in this study, new ways of combining other cancer biomarkers should be considered, like microchips as a multiplexed platform. In addition, EM or AFM images for different functionalization conditions would reveal new insights on the surface morphology changes that affect the higher sensitivity of protein G-treated SDIs and become strong support for the hypothesis.

In addition, there was already successful detection of HER2 and CD44 biomarkers in our laboratory. There is a possibility to combine two or more biomarkers for the detection of a specific type of cancer.

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