Using the dynamic platform in biosensing cancer biomarker CD44

Zhuldyz Myrkhiyeva

School of Engineering and Digital Sciences
Department of Chemical and Materials Engineering

Supervisor: Associate Professor Daniele Tosi
Co-supervisor: Assistant Professor Chang-Keun Lim

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- Conclusion
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Introduction

- Cancer cases is predicted to be **28.4 million** in 2040 [1].
- In Kazakhstan, cancer is the second leading cause of death after cardiovascular diseases. Due to a lack of medical equipment for diagnosis and treatment[2].
- Cancer metastasis is responsible for more than **90%** of cancer-related death [3].
- **CD44** is one of the proteins that play a significant role in cancer metastasis [4].

Table 1. Significance and function of CD44 [4].

CD44 isoforms	Biological functions	Cancer types
CD44s	Tumor growth, metastasis, low survival rate	Pancreatic cancer
CD44s	Progression	Breast cancer
CD44v3	Migration, overexpressed in tumor tissue	Head and neck squamous cell carcinoma
CD44v3	Proliferation and cisplatin resistance	Head and neck squamous cell carcinoma
CD44v4, CD44v5	Lung metastasis loci	Pancreatic cancer
CD44v6	Metastasis; association with liver metastasis	Pancreatic cancer
CD44v6	Epithelial phenotype cells expression	Prostate cancer
CD44v6	Tumorigenic and chemoresistance	Prostate cancer
CD44v6	Migration, metastasis, advanced stage of tumor	Colorectal adenocarcinomas
CD44v6	Metastasis	Colon cancer
CD44v6,CD44v9	Correlates with lymph node metastasis, liver metastasis, and TNM stage	Pancreatic cancer
CD44v9	Lower survival rate, correlates with lymph node/liver metastasis, and TNM stage	Pancreatic cancer
CD44v9	Associated with worse prognosis, contributed to EMT-mediated invasion and migration	Bladder cancer
CD44v9	Inhibited assembly of p-cMet, AR, HSP90, P110α/PI3K, and CD44 into lipid raft like structures	Prostate cancer
CD44v4-10	Promoted adenoma initiation in Apc(Min/+)mice, tumor initiation	Colorectal cancer
CD44v8-10	Tumor initiation	Gastric cancer
CD44v8-10	Lung metastasis	Breast cancer
CD44v (v6-10,v7-10,v8-10)	Correlative study of CD44v expression on transgenic Gan mice	Gastric tumor
CD44v8-10 overexpression	Enhance chronic phase CML progenitor replacing capacity	Leukemia
CD44, isoforms not specified	Correlative study of CD44 expression on malignant stage	Prostate cancer
CD44, isoforms not specified	Increased colony formation, invasion	Prostate cancer
CD44, isoforms not specified	Poor prognosis, low survival rate, metastasis	Pancreatic cancer

Importance of CD44

- Cluster of differentiation 44 (CD44): important tumor protein associated with metastasis [4].
- Overexpressed on the cell surface in **cancer stem cells** [5].
- **CD44 protein** is in soluble form found in serum [6].
- CD44 is a **prognostic** and **diagnostic** biomarker for several types of cancer [6].
- Overexpression of CD44 in the human body signals tumor progression [4].

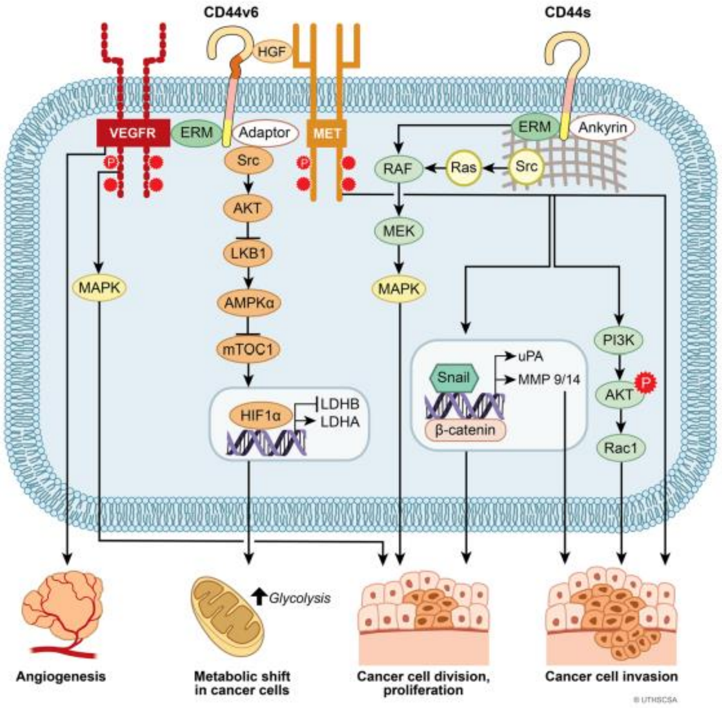

Figure 1. CD44-mediated downstream signaling pathways [4].

Significance of the study

CD44 detection methods

- ELISA : Commercially available, time consuming, not label free
- OncAlert: fast but not label free
- Electrochemical and photo-electrochemical biosensors: can not be used *in vivo*

Problem:

Accurate, easy to use CD44 biomarker detection method is needed that can help in the improvement of cancer treatment outcome.

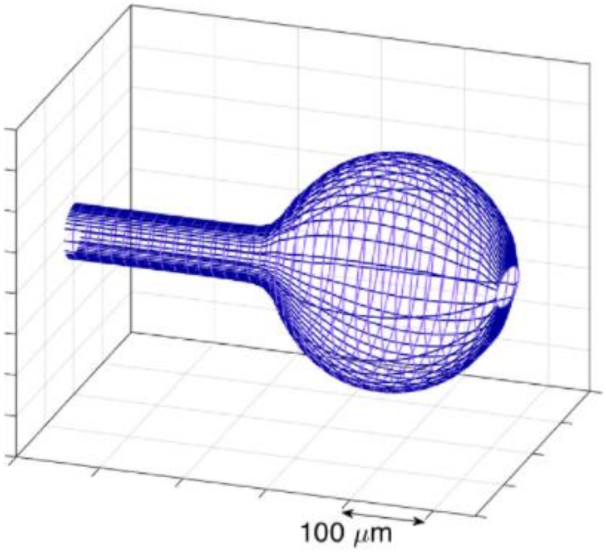

Figure 2. 3D image of ball resonator created from profilometry data [8]

Hypothesis and Aims

Hypothesis:

Biocompatible, sensitive, label free, optical fiber biosensor with a small size, low cost is a good candidate for CD44 biomarker detection.

Aims and Objectives:

Construction of an accurate biosensing platform that detects cancer biomarker CD44 in a dynamic setup using a fiber optic sensor fabricated in the lab.

The following objectives are pursued in this regard:

- Fabrication of the sensor
- Calibration, functionalization of sensor
- Building *in-vitro* platform
- Protein measurements

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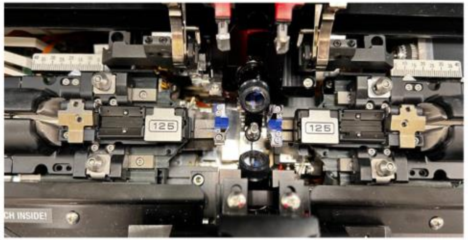
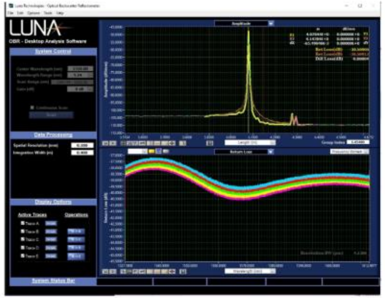
Methodology

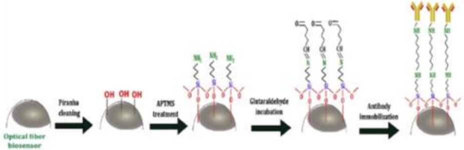
1 Sensor Fabrication



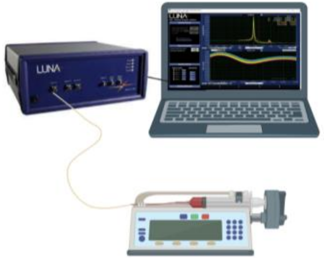
2 Calibration



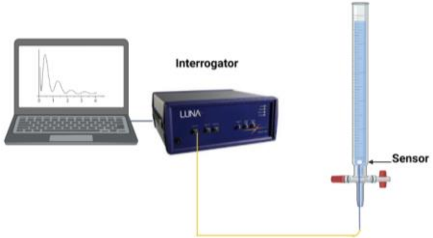
3 Biosensor Functionalization



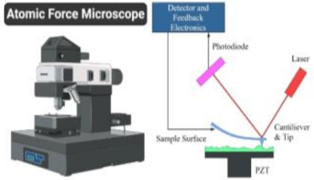
4 Protein Measurement



5 Pressure Characterization



6 Surface Morphology Study



Methods: Optical fiber ball resonator fabrication

Figure 3. Fujikura LZM-100 machine (A) with interaction window (B) and single mode fiber placing area (C).

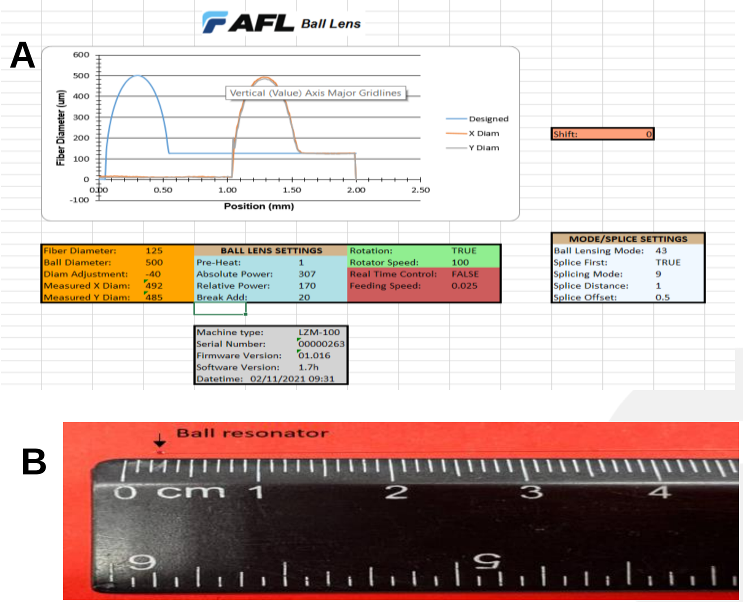


Figure 4. Created report by FPS when ball resonator was successfully fabricated (A). The real image of sensor.

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Methods: Sensor calibration and functionalization

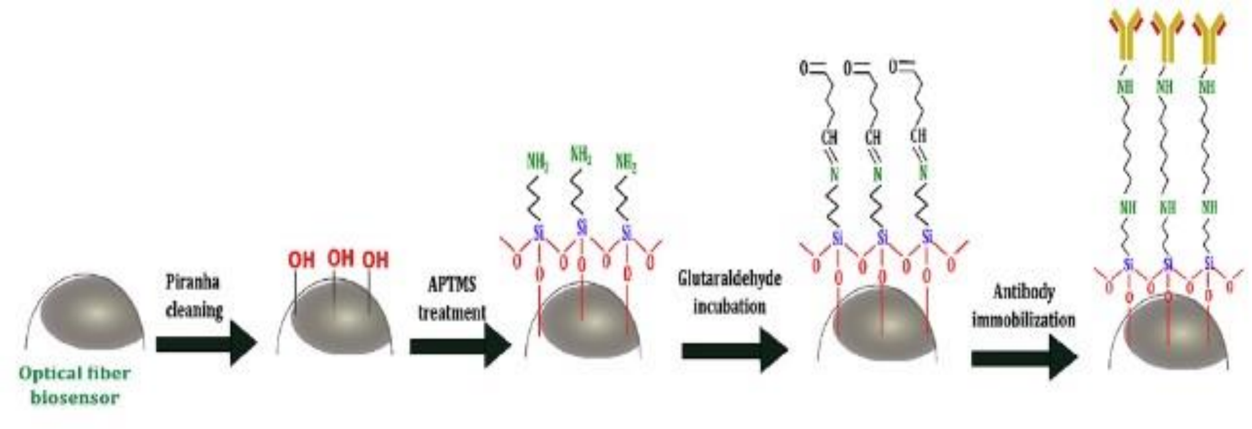


Figure 5. Optical fiber ball resonator surface functionalization [9].

Methods: proteins measurements

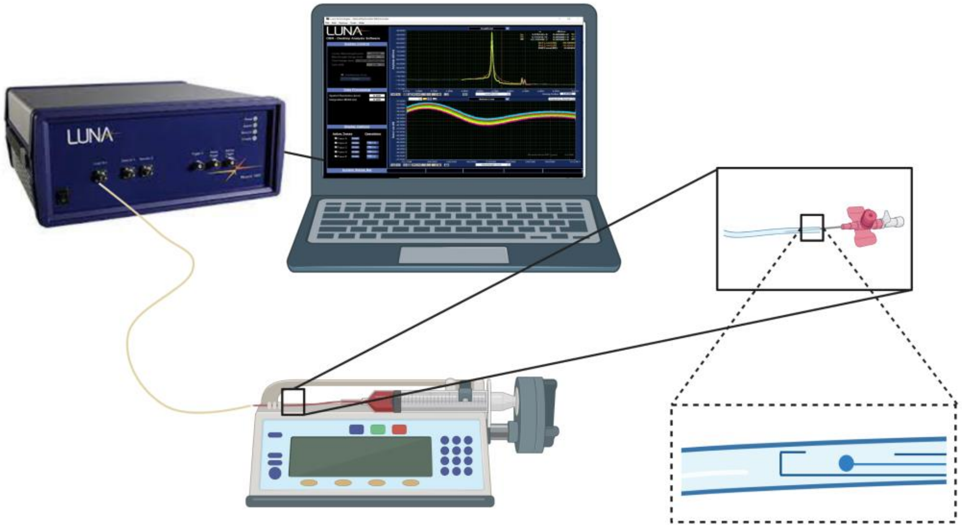


Figure 6. In-vitro CD44 and control proteins measurement using dynamic setup.

Pressure characterization and surface morphology study

Figure 7. Pressure measurement study.

Results and Discussion

Table 2. Optical fiber ball resonators used in dynamic setup.

	Diameter (µm)	Reflection dB/mm	Sensitivity (dB/RIU)	R^2	At the wavelength (nm)	Purpose
1	489-492	air:35 water:47	-96	1,00	1 565,42	Gamma globulin detection
2	491-485	air:35 water:48	-90	0.96	1 552,75	CD44 detection
3	492-485	air:37 water:47	-98	0,95	1 573,42	CD44 detection
4	496-491	air:35 water:45	-83	0,99	1 552,98	CD44 detection
5	497-492	air:35 water:50	-98	0,95	1 571,60	CD44 detection
6	497-491	air:36 water:48	-74	0,99	1 553,14	Pressure measurement
7	499-494	air:36 water:48	-85	0,97	1 552,85	CD44 detection
8	521-515	air:37 water:49	-128,37	0,98	1 552,55	CD44 detection
9	533-527	air:36 water:46	-105	0,97	1 580,55	CD44 detection
10	485-491	air:37 water:50	-83	0,98	1 552,88	Thrombin detection
11	518-510	air:44 water:54	-105	0,95	1 565,42	Thrombin detection
12	499-494 (1)	air:36 water:48	-84	0,97	1 570,99	Pressure measurement

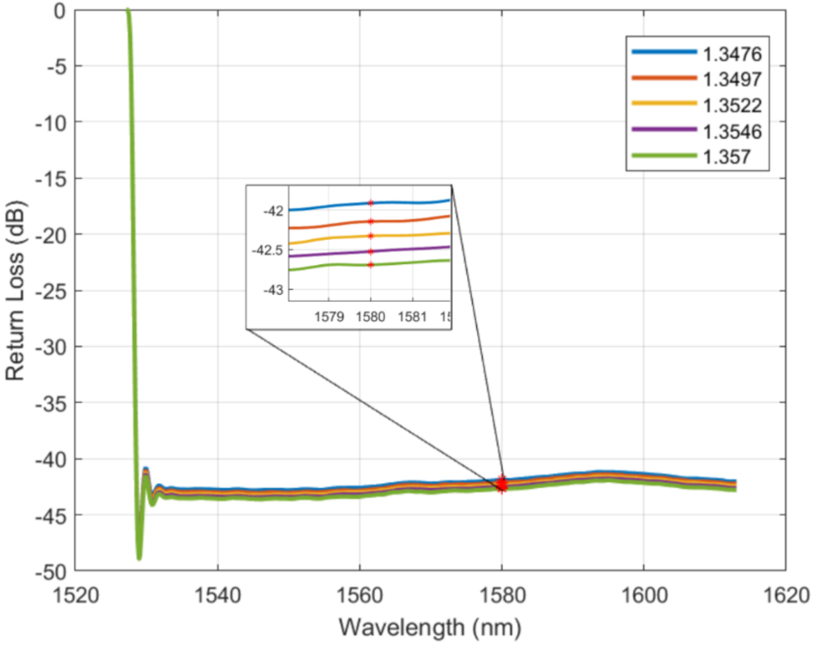


Figure 8. RI calibration of the sensor.

In-vitro measurements

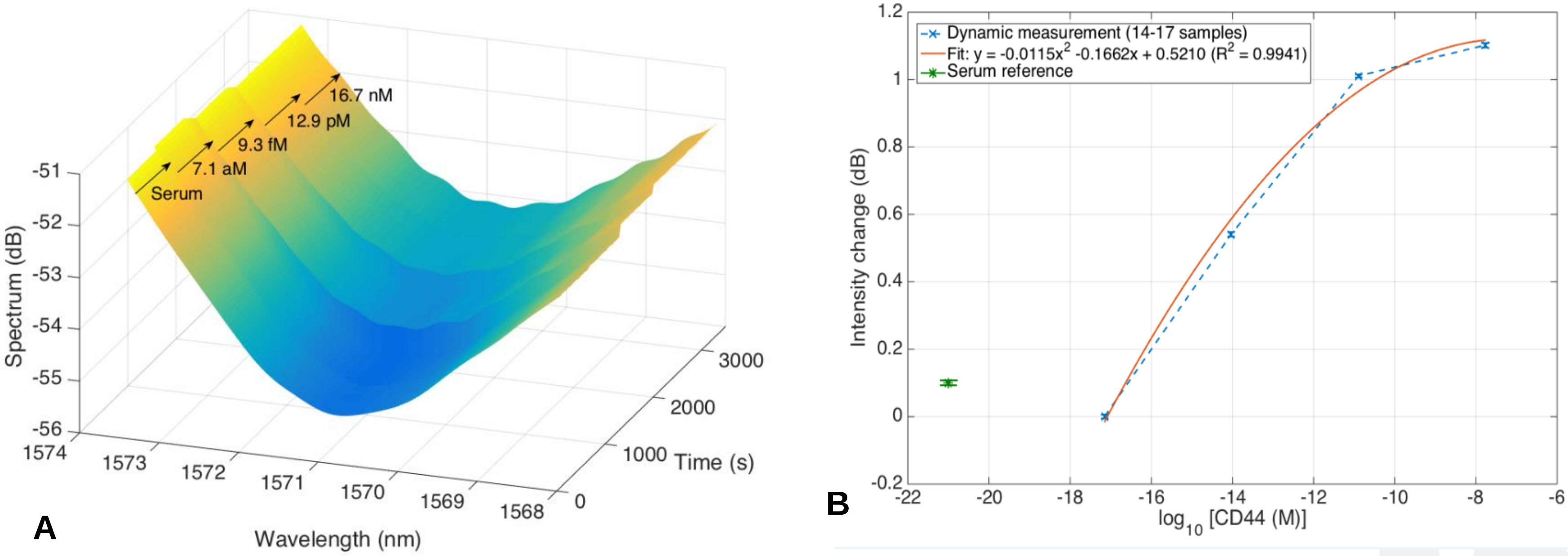


Figure 9. In-vitro CD44 detection (A). Intensity change as a function of protein concentration (B).

Repeatability results

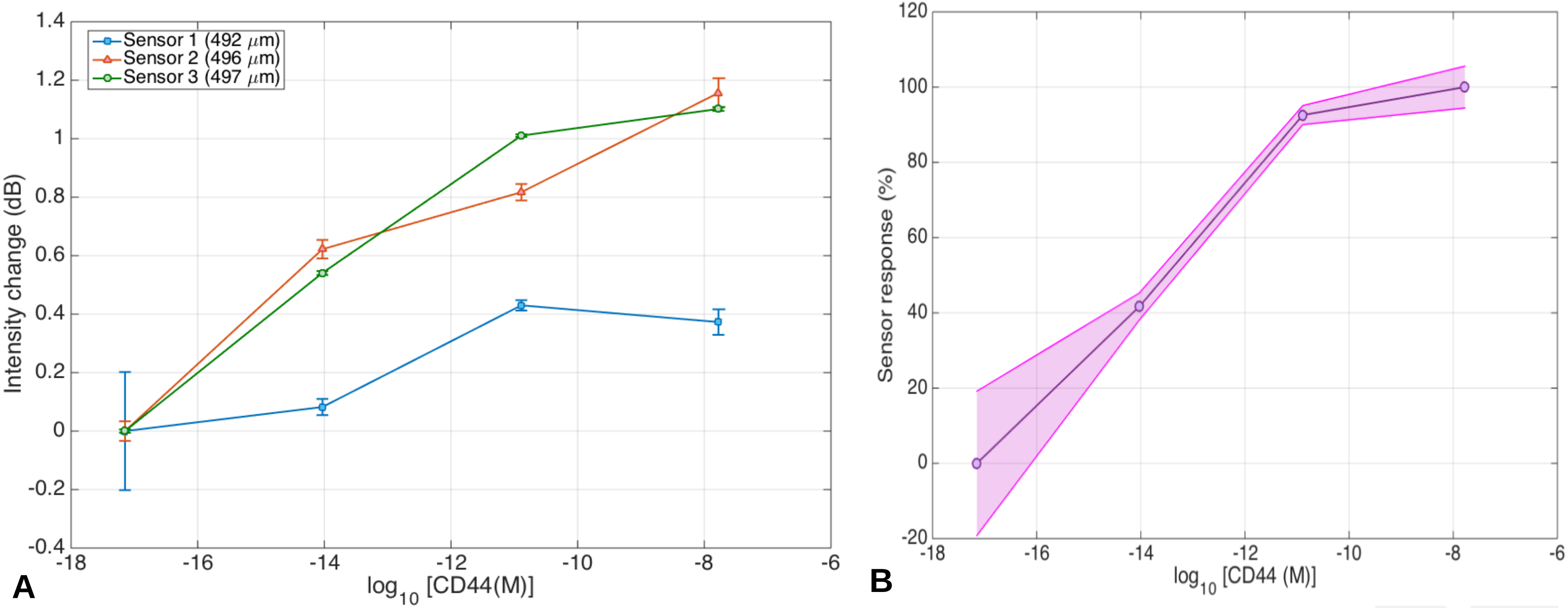


Figure 10. Reproducibility test results for 3 sensors (A). Solid line = average from three different sensors; shaded region = range between minimum and maximum of response (B).

Specificity studies

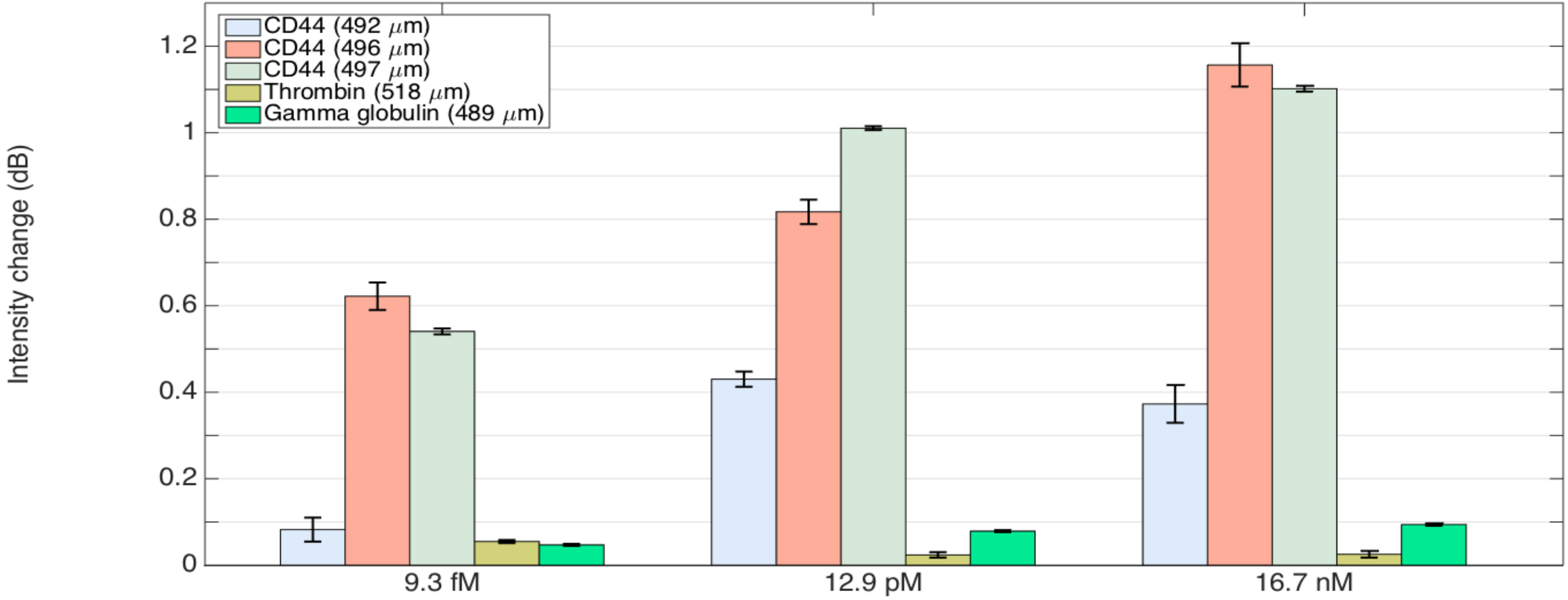


Figure 11. Evaluation of the specificity and quality of functionalization for the CD44 biosensor.

Pressure sensitivity

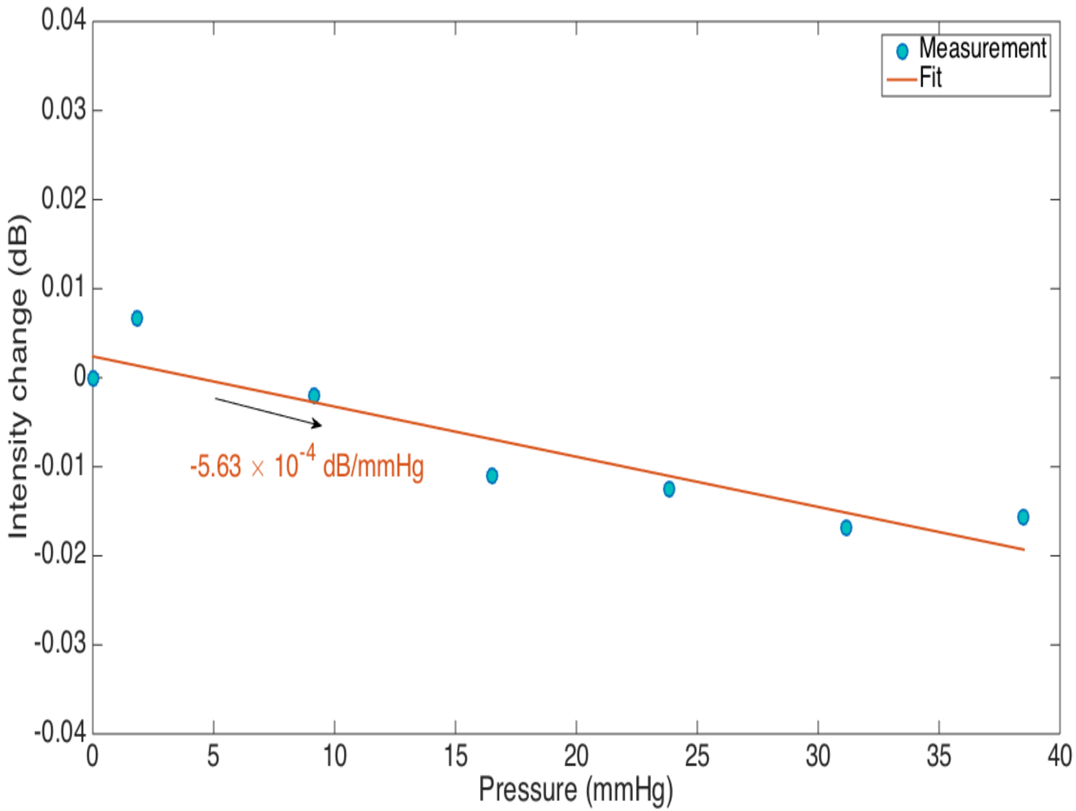


Figure 12. The pressure measurement of ball resonator with a diameter of 499-494 µm.

AFM analysis

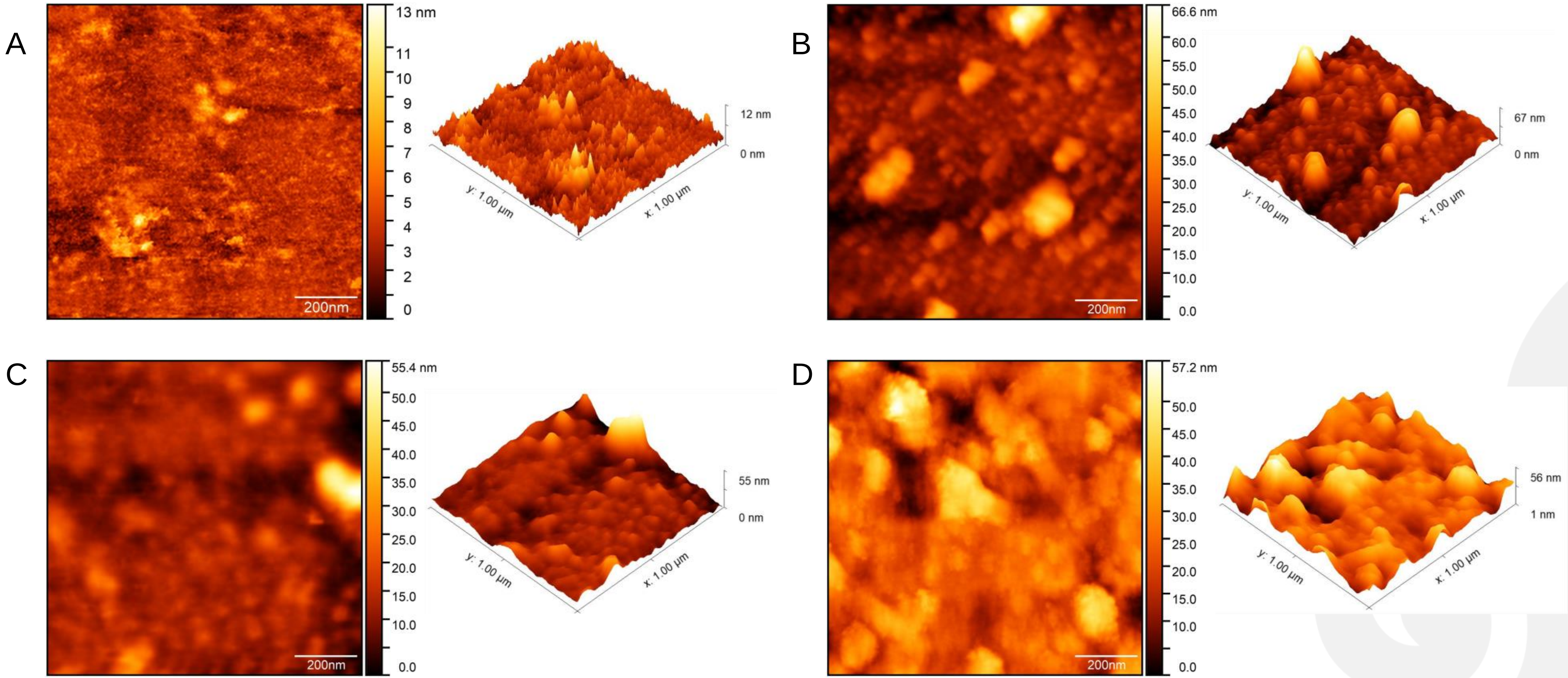
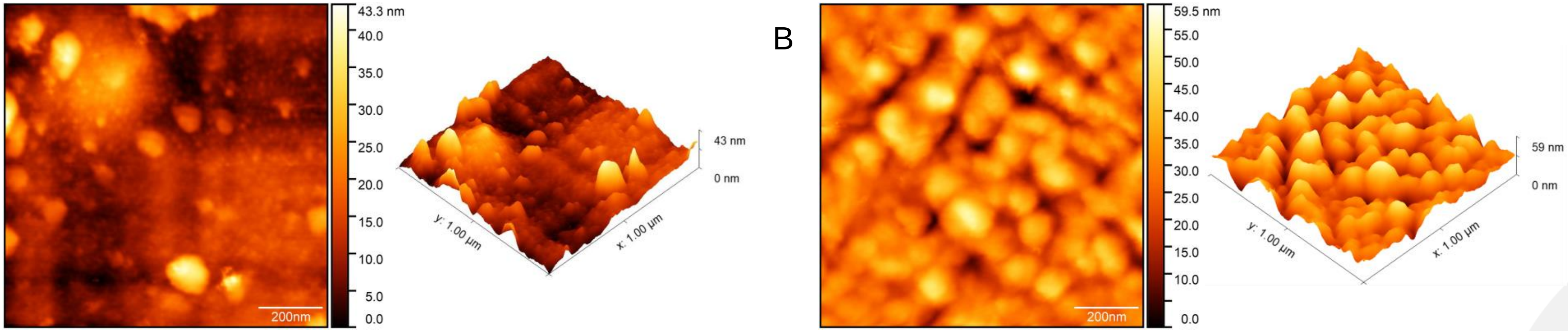


Figure 13. AFM height images and 3D images of sensors at different stages of functionalization.
After treatment in Piranha solution (A), APTMS (B), Heat treatment after APTMS (C), Glutaraldehyde 25%(D).

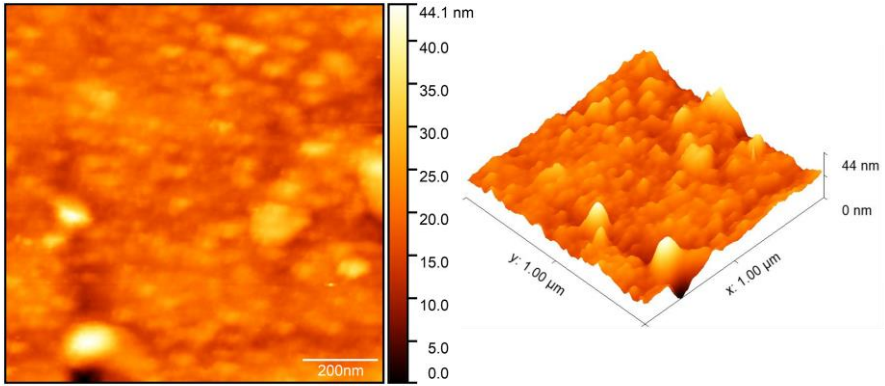
AFM analysis

A

B



C



Treatment	Piranha	APTMS	Heat	GA	Ab	mPEG	Ball resonator
Height, nm	18.97±1.164	60.36±4.947	61.50±3.16	58.86±3.24	40.24±2.67	54.45±2.56	51.45±3.65
RMS roughness, nm	1.531±0.124	7.224±0.624	8.468±0.50	6.774±0.51	4.703±0.35	7.824±1.03	6.197±0.56

Figure 14. AFM height images and 3D images of sensors at different stages of functionalization. After treatment Antibodies (A), m-PEG (B), CD44 protein (C).

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Conclusion

- *In vitro* setup that imitated the blood flow was optimized.
- Optical fiber biosensor was able to detect CD44 in dynamic condition.
- Reproducibility studies were done.
- Optical fiber biosensor was specific towards CD44.
- Optical fiber biosensor was pressure insensitive.

Limitations:

- Availability of optical fiber ball resonators due to equipment issues
- Bubble formation during *in-vitro* measurement

Future work:

- Hand model building with skin material
- More complex media
- Measurement of CD44 expressing cell with optical fiber biosensor, moving towards more complex analyte
- Optimization of biosensor functionalization with hyaluronic acid, natural ligand

The output of the work

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3) Aliya Bekmurzayeva, Zhannat Ashikbayeva, **Zhuldyz Myrkhiyeva**, Aigerim Nugmanova, Madina Shaimerdenova, Takhmina Ayupova, Daniele Tosi. "Label-free fiber-optic spherical tip biosensor to enable picomolar-level detection of CD44 protein." Scientific reports 11 (1), 19583, **2021**

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