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# Detection of cardiac troponin-I by optic biosensors with immobilized anti-cardiac troponin-I monoclonal antibody

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ABSTRACT

In this study, it is aimed to determine cardiac troponin I by a surface plasmon resonance biosensor immobilized anti-cardiac troponin I monoclonal antibody. The immobilized anti-cardiac troponin I monoclonal antibody surface plasmon resonance biosensors were characterized with ellipsometry, atomic force microscopy and contact angle analysis. After that, surface plasmon resonance biosensor system was completed to biosensor system to investigate kinetic properties for cardiac tropinin I. The sensing ability of surface plasmon resonance biosensor was investigated with 0.001–8.0 ng/mL concentrations of cardiac tropinin I solutions. The limit of detection and limit of quantification were calculated as 0.00012 ng/mL and 0.00041 ng/mL, respectively. To show the selectivity of surface plasmon resonance biosensor competitive adsorption of cardiac tropinin I, myoglobin, immunoglobulin G and prostate specific antigen were investigated. Surface plasmon resonance biosensor was investigated five times with 0.5 ng/mL concentrations of cardiac tropinin I solution to show reuse of the chip. The results showed that surface plasmon resonance biosensor has high selectivity for cardiac tropinin I. The reproducibility of surface plasmon resonance sensors was investigated both on the same day and on different days for five times. To determine the usability, selectivity and validation studies of surface plasmon resonance biosensors were performed by enzyme-linked immunosorbent assay method.

## 1. Introduction

One of the most common causes of death is ischemic heart disease. Because of the high mortality and morbidity of myocardial infarction due to ischemic heart disease, rapid and reliable diagnosis is vital in clinical analysis [1,2]. Examinations and clinical findings used in the diagnosis of the disease do not always give reliable results. Because of these reasons, proteins with increased amounts in the blood circulation due to cardiac injury can be used as biomarkers for early diagnosis. Cardiac troponins, the specific biomarker for myocardial tissue, consists of cardiac troponin I (cTnI) and troponin T (cTnT). For myocardial infarction, cardiac troponin I and troponin T are more specific and sensitive than other cardiac biomarker such as creatine-kinase and myoglobin [3]. One of the commonly used cardio specific biomarkers is cardiac troponin I (cTnI) [4-7]. Cardiac troponin I (cTnI) is a 23 kDa protein consisting of 210 amino acids with isoelectric point (pI) of 9.87 [8]. Cardiac troponin I (cTnI) used as acute myocardial infarction in the early diagnostic. The significant range of cardiac troponin I (cTnI) is 0.001–0.1 ng/mL in the blood [9]. The level of cTnI concentration is around 0.001 ng/mL in normal patients. The level of cTnI concentration is around 0.1 ng/mL in acute heart failure [10]. Cardiac troponin I (cTnI) has become increased important in the blood. There is a need for efficient, simple and rapid methods for detection of cardiac biomarkers [11,12]. Surface plasmon resonance (SPR) biosensors offer the advantage of rapid, label-free simultaneous monitoring of binding events due to refractive index changes near the biosensor surface. SPR biosensors have a unique technology that enables label free and direct observation of biomolecular interaction in real-time [13]. SPR biosensors have a wide range of applications in multiple fields such as immunoassay, clinical analysis and diagnosis of disease, pharmaceutics, screening for disease, industrial processing, monitoring and environmental pollution control, veterinary, food and agricultural applications [14-24].

Compared to classical analytical methods such as chromatography methods, chemiluminescent enzyme immunoassay, radioimmunoassay applications [25-27], surface plasmon resonance biosensors have advantages such as real-time monitoring, rapid, analysis time, sensitivity and low cost. Classical analytical methods are used for diagnostic analysis but which is high cost equipment, multistep processing, time-consuming and need skilled person during the analysis. Therefore,

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selective, sensitive and rapid detection methods should be used for detection of cardiac troponin I (cTnI).

In this study, we chosen a cardio-specific biomarker as a model cardiac troponin I (cTnI). We aimed to develop a highly sensitive selectivity surface plasmon resonance (SPR) device for screening of cardiac troponin I (cTnI). Firstly, several activators were used for the gold chip surface modification. After, anti-cardiac troponin I monoclonal antibody was immobilized onto the modified gold surface of the SPR chip. In this study, it was observed that the immobilization of specific anti-cardiac troponin I monoclonal antibody for cardiac troponin I was increased the selectivity of the SPR biosensor. SPR biosensor was prepared with anti-cardiac troponin I monoclonal antibody which was immobilized onto the modified gold surface of the SPR chip. The immobilization of monoclonal antibody onto the SPR biosensors was characterized with ellipsometry, atomic force microscopy and contact angle analysis. The kinetic studies of cardiac troponin I (cTnI) were applied with the use of aqueous cardiac troponin I (cTnI) solutions in 0.001–8.0 ng/mL concentrations. Accordingly, kinetic binding analyzes were performed with SPR system. Limit of detection (LOD) and limit of quantification (LOQ) were calculated with using kinetic analysis data. The selectivity studies of SPR biosensors were determined with myoglobin (Mb), immunoglobulin G (IgG) and prostate specific antigen (PSA). To determine the usability and selectivity of SPR biosensor, patient serum samples were collected from Numune Training and Research Hospital, Ankara, Turkey. Validation studies of surface plasmon resonance biosensors were carried out by enzyme-linked immunosorbent assay (ELISA) method.

## 2. Experimental

### 2.1. Materials

The template molecule, cardiac troponin I antigens, monoclonal cTnI antibodies, myglobin, immunoglobulin G and prostate specific antigen were obtained from Sigma Chemical Co. (St. Louis, USA). Poly (acrylic acid) (PAA), 1-(3-Dimethylaminopropyl)-3-ethalcarbodimide hydrochloride (EDC), poly-(diallyldimethylammonium chloride) (PDDA), N-Hydroxysuccinimide (NHS), phosphate buffer saline (PBS), potassium hydroxide (KOH) and bovine serum albumin (BSA) were purchased from Sigma Aldrich (StLouis, USA). SPR gold chips (SPRchipTM) were obtained from GenOptics, SPRiLab (Orsay, France).

### 2.2. Modification steps of SPR biosensor surface

Details of the immobilization of anti-cardiac troponin I monoclonal antibody onto the SPR biosensor surfaces was reported elsewhere [28]. Before the surface modification of the SPR chip, the gold surface was washed with purified water and dried with air. The SPR gold chip surface was modified layer-by-layer with several activators. To create hydroxyl(-OH) groups on the chip surface, the surface was incubated with 0.1 M KOH solution for 10 min. The gold chip was cleaned with purified water. To get final modification with capture proteins, SPR biosensor surface was prepared by incubation for 30 min and 2 mg/mL poly(acrylic acid) (PAA), (negatively charged) and 2 mg/mL poly(diallyldimethylammonium chloride) (PDDA) (positively charged). The SPR biosensor surface was cleaned with purified water. 100 µL of N-hydroxysuccinimide (NHS, 50 mM) and N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC, 100 mM) mixture were introduced and incubated for 30 min. EDC reacts with carboxyl groups to form an amine reactive intermediate that is stabilized by adding NHS. To reduce subsequent non-specific binding, 3% BSA in pH 7.4 soultion was introduced and incubated SPR biosensor surface for 30 min. The gold chip was cleaned with purified water. Finally, functionalized SPR biosensor was incubated with anti-cardiac troponin I monoclonal antibody with concentration of 100 µg/mL for 1 h at room temperature. All the steps were monitered in real-time on the SPR system. The immobilization of anti-cardiac troponin I monoclonal antibody onto the SPR biosensor surfaces and the detection process were shown in Fig. 1.

### 2.3. Surface characterization SPR biosensor

Ellipsometer, contact angle and atomic force microscopy measurements were made for the characterization of SPR chip surfaces. Ellipsometry measurements (Nanofilm EP3, Goetting, Germany) were performed by using an auto-nulling imaging ellipsometry [29]. The characterization of SPR biosensors was carried out by using AFM in tapping mode (Nanomagnetic Instruments, Oxford, UK) [30]. AFM takes measurements in high resolution (i.e., 4096 x 4096). Sample was scanned at the scanning rate of 2 µm/s and 256 x 256 pixels resolution to obtain view of 2 µm × 2 µm area. The thickness measurements have been done at a wavelength of 658 nm with an angle of incidence 65°. Contact angle (KRÜSS DSA 100, Hamburg, Germany) of the SPR biosensor was measured with sessile drop method with the using water [31]. The contact angle measurements were performed to determine the hydrophilicity/hydrophobicity of SPR biosensor surface.

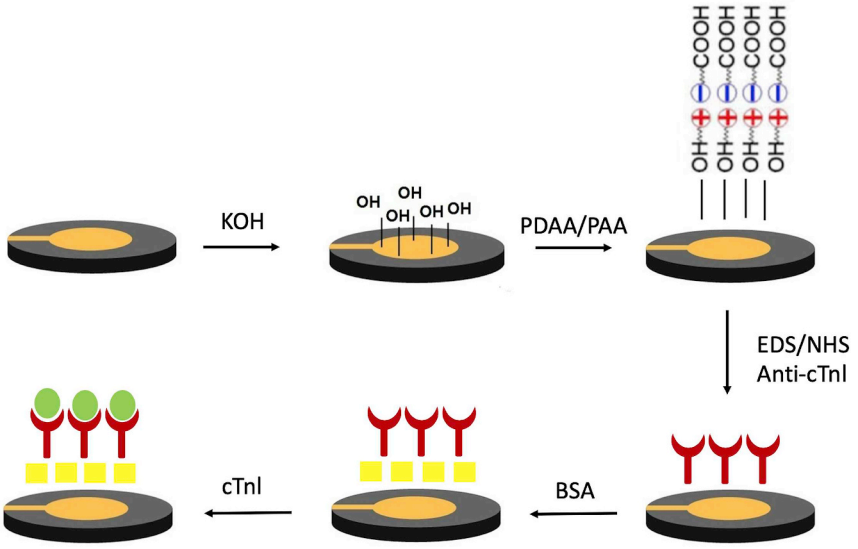


**Fig. 1.** Schematic illustration of SPR biosensor surface modification for detection of cardiac troponin I (cTnI).

### 2.4. Kinetic analysis

Before the kinetic analysis, the gold chip surface modification was optimized. The SPR analysis was real-time monitored on the SPR system (GenOptics, SPRiLab, Orsay, France). The immobilization of antibody on the SPR biosensor was used for the real-time detection of cardiac troponin I from aqueous solution. Firstly, the SPR biosensor was washed with purified water and pH 7.4 buffer solution. Then cardiac troponin I (cTnI) solutions in 0.001–8.0 ng/mL concentrations were applied to the SPR system. The signal was real-time monitored on the SPR system. Finally, the SPR biosensor was washed with purified water and pH 7.4 buffer solution.

To determine the isotherm parameters, three different isotherm models as a Langmuir, Freundlich, and Langmuir-Freundlich were calculated for SPR biosensor [32]. The interaction model between SPR biosensor and cardiac troponin I can be described by the below equations:

$$\textbf{Langmuir} \quad \Delta R = \Delta R_{max} C / K_D + C \tag{1}$$

$$\textbf{Freundlich} \quad \Delta R = \Delta R_{max} C^{1/n} \tag{2}$$

$$\textbf{Langmuir} - \textbf{Freundlich} \quad \Delta R = \Delta R_{max} C^{1/n} / K_D + C^{1/n} \tag{3}$$

The resonance frequency change is ΔR, which measure signal by binding of cardiac troponin I (cTnI); C (ng/mL) is concentration of cardiac troponin I (cTnI). $K_D$ (mL/ng) is the dissociation equilibrium constant. Also, association equilibrium constant, $K_A$ (ng/mL), may be calculated $1/K_D$. Freundlich exponent is described with 1/n. The maximum subscript refers max [33,34].

The real-time detections of cardiac troponin I (cTnI), myoglobin (Mb) immunoglobulin G (IgG) and prostate specific antigen (PSA) solutions in Mb, IgG, PSA solution and mixed (Mb + IgG + PSA) solution in competitive manner were analyzed in order to determine the specificity of SPR biosensor. The selectivity of SPR biosensors was examined at 0.5 ng/mL of each a protein concentrations.

### 2.5. Reproducibility and stability studies

The reproducibility of SPR biosensors was realized by equilibration-binding-regeneration cycles for several times. The reproducibility of the SPR biosensor was tested on the same day and on the different days. Reusability of SPR biosensor was repeated 5 times at 0.5 ng/mL concentrations of cardiac troponin I (cTnI). The signal was real-time monitored on the SPR system. SPR signal responses were recorded as a percentage of the change in reflectivity (%ΔR) and used for the calculation of the reproducibility and storage stability. %ΔR is the resonance frequency change in the SPR biosensor.

### 2.6. Clinical analysis

Patient serum samples provided important information about the selectivity and reproducibility of the SPR biosensor system. To determine the selectivity and the validation of the SPR system, patient serum samples were collected from Numune Training and Research Hospital, Ankara, Turkey. Informed consent was obtained from patients under troponin follow up for dual sampling and protocol was approved by the administration. Patient serum samples were centrifuged at 3000 rpm for 20 min and these samples were stored in a deep freeze at −20 °C. Patient serum samples were diluted with 10 mM phosphate buffer (pH 7.4) in the ratio of 1/4 (v/v). Firstly, the SPR biosensor was washed with purified water and pH 7.4 buffer solution. The diluted patient serum samples were applied to the SPR system (25 mL, 0.5 mL/min, at room temperature). The signal was real-time monitored on the SPR system. Finally, the SPR biosensor was washed with purified water and pH 7.4 buffer solution. Results were compared with the results obtained from ELISA measurements performed in Numune Training and Research Hospital, Ankara, Turkey.

## 3. Results

### 3.1. Surface characterization of the SPR biosensors

For the morphology studies of SPR chip surfaces were used ellipsometry, contact angle and atomic force microscopy (AFM) analysis. Ellipsometry measurements were performed to obtain the data about SPR chip surface. Surface depth on the SPR biosensor surface from ellipsometry was obtained as 42.8 nm of the modified SPR chip surface in Fig. 2A.

Contact angle measurements of the SPR biosensor surfaces were measured with sessile drop method with the using water. The contact angle values of SPR chip surface were decreased from 82.7° to 69.8° of the clean SPR chip surface and modified SPR biosensor surface, respectively. This data in contact angle value of the chip surface stemmed from increased hydrophilic structure of the chip surface in Fig. 2B and C.

AFM image of the chip surface was given in Fig. 2D and E. As can be clearly seen from the image, it is seen that polymeric surface attached to the SPR chip is formed by modification steps. As seen in the Fig. 2D and E, the surface deepness of the unmodified SPR chip and modified SPR chip surfaces were determined as 9.68 nm and 34.62 nm, respectively.

### 3.2. Kinetic studies

The immobilization of anti-cardiac troponin I monoclonal antibody onto the SPR biosensor surfaces was demostrated step-by-step in Fig. 3. The SPR gold chip surface was modified layer-by-layer with several activators. To create hydroxyl (-OH) groups on the SPR chip surface, the surface was incubated with 0.1 M KOH solution. To get final modification with capture proteins, SPR biosensor surface was incubated with PAA (negatively charged) and PDDA (positively charged). After, SPR biosensor surface was incubated with NHS/EDC mixture. EDC reacts with carboxyl groups to form an amine reactive intermediate that is stabilized by adding NHS. To reduce subsequent non-specific binding, 3% BSA in pH 7.4 soultion was incubated SPR biosensor surface. Finally, functionalized SPR biosensor was incubated with anti-cardiac troponin I monoclonal antibody. All the steps were monitered in real-time on the SPR biosensor system with observing the refractive index changes (ΔR).

In kinetic studies, cardiac troponin I (cTnI) samples were prepared in pH 7.4 phosphate buffer solutions at 0.001–8.0 ng/mL concentrations. The sensorgrams were real-time monitored on the SPR system and were shown in Fig. 4. Firstly, the SPR biosensor was washed with purified water and pH 7.4 buffer solution for 5 min. The SPR biosensor was interacted with aqueous cardiac troponin I (cTnI) solutions in different concentrations in the range of 0.001–8.0 ng/mL (Fig. 4A). As seen from figure, equilibration-adsorption-regeneration cycles for a new analysis were run in 13.30 min for cardiac troponin I (cTnI). As the cardiac troponin I (cTnI) concentration increases, the %ΔR value is clearly increased in Fig. 4B. The good linear equation between 0.001 and 8.0 ng/mL concentration is y = 11.6x + 0.9117 with a coefficient of determination of 0.998.

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated from the slope of the calibration curve of cardiac troponin I (cTnI) molecules. Limit of quantification (LOQ) and limit of detection (LOD) were calculated with the equation [35].

$$LOQ = 10\,S/m \tag{4}$$

$$LOD = 3.3\,S/m \tag{5}$$

where S is the standard deviation of the intercept and m is the slope of the regression line.

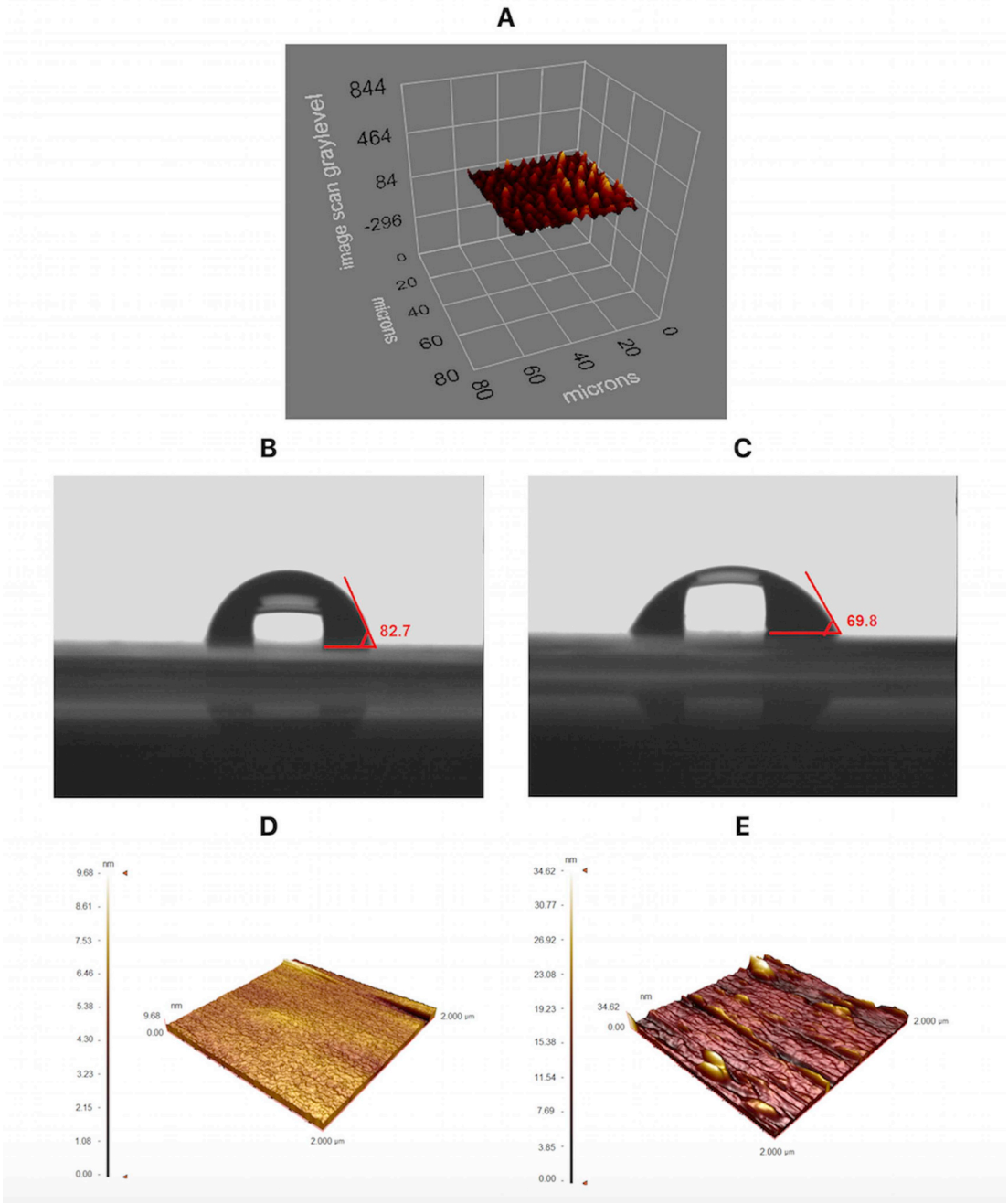


**Fig. 2. Ellipsometry image (A:** modified SPR chip surface), **Contact angles** (**B:** unmodified SPR surface, **C:** modified SPR chip surface), **AFM images** (**D:** unmodified SPR surface, **E:** modified SPR chip surface).

Chen et al. [36] prepared an immunomagnetic separation technology-assisted surface plasmon resonance biosensor for human cardiac troponin I (cTnI). In consequence of the experiments, the detection limit for cardiac troponin I (cTnI) was calculated as 3.75 ng/mL. Jo et al. [37] developed an electrochemical aptasensor of cardiac troponin I (cTnI) in human serum albumin solution. In this study, a linear range was observed 1–10.000 pM in a buffer and detection limit of 1.0 pM (24 pg/mL). Negahdary et al. [38] designed an electrochemical aptasensing for the detection of cardiac troponin I (cTnI). The limit of detection in the linear concentration range from 1 0.05 ng/mL to 500 ng/mL was calculated as 8.0 pg/mL. In our study, limit of detection (LOD) and the limit of quantification (LOQ) in the linear concentration range from 0.001 ng/mL to 8.0 ng/mL were calculated as 0.00012 ng/mL and 0.00041 ng/mL, respectively. The comparative other studies for detection of cardiac troponin I (cTnI) in the literature were also given in Table 1.

In order to detect the isotherm parameters, SPR biosensor data were examined using three different isotherm models (such as Langmuir, Freundlich and Langmuir-Freundlich). Langmuir, Freundlich and Freundlich-Langmuir isotherm models were given in Table S1. Langmuir model was realized bounding sites cardiac troponin I (cTnI) for SPR chip surfaces owing to the obtained data in Figure S1. For SPR biosensors, the linearity of the Langmuir isotherm model was better than that of Freundlich and Langmuir–Freundlich isotherm models. However, especially the Langmuir model with $\Delta R_{max}$ equals 3.235 is found to be the most appropriate model to identify the interaction between SPR biosensor and cardiac troponin I (cTnI) molecules.

Selectivity of SPR biosensor was determined by cardiac troponin I (cTnI), myoglobin (Mb), immunoglobulin G (IgG) and prostate specific antigen (PSA) solutions in Mb, IgG, PSA solution and mixed

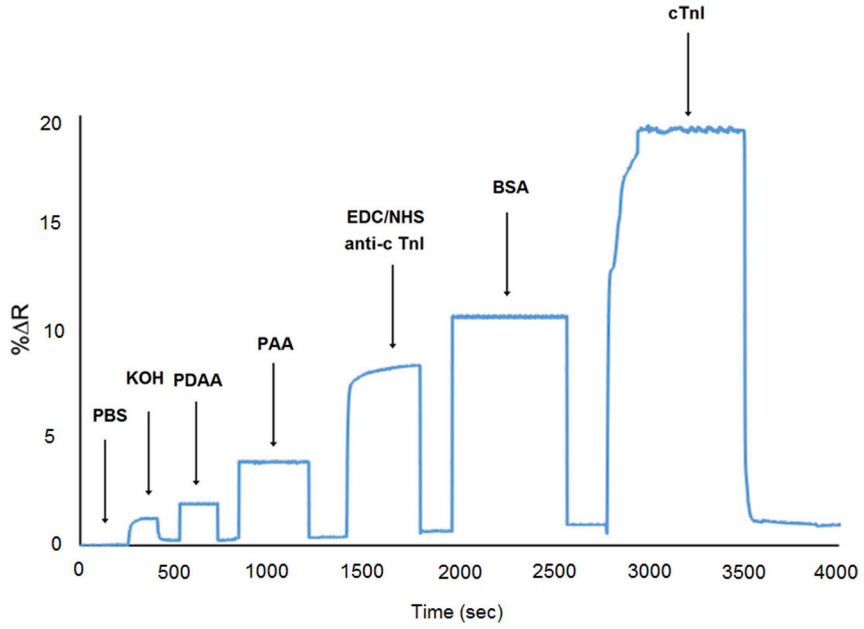


**Fig. 3.** The surface modification of SPR biosensor chip surface ($V_{solution}$: 5 mL, T: 25 °C).

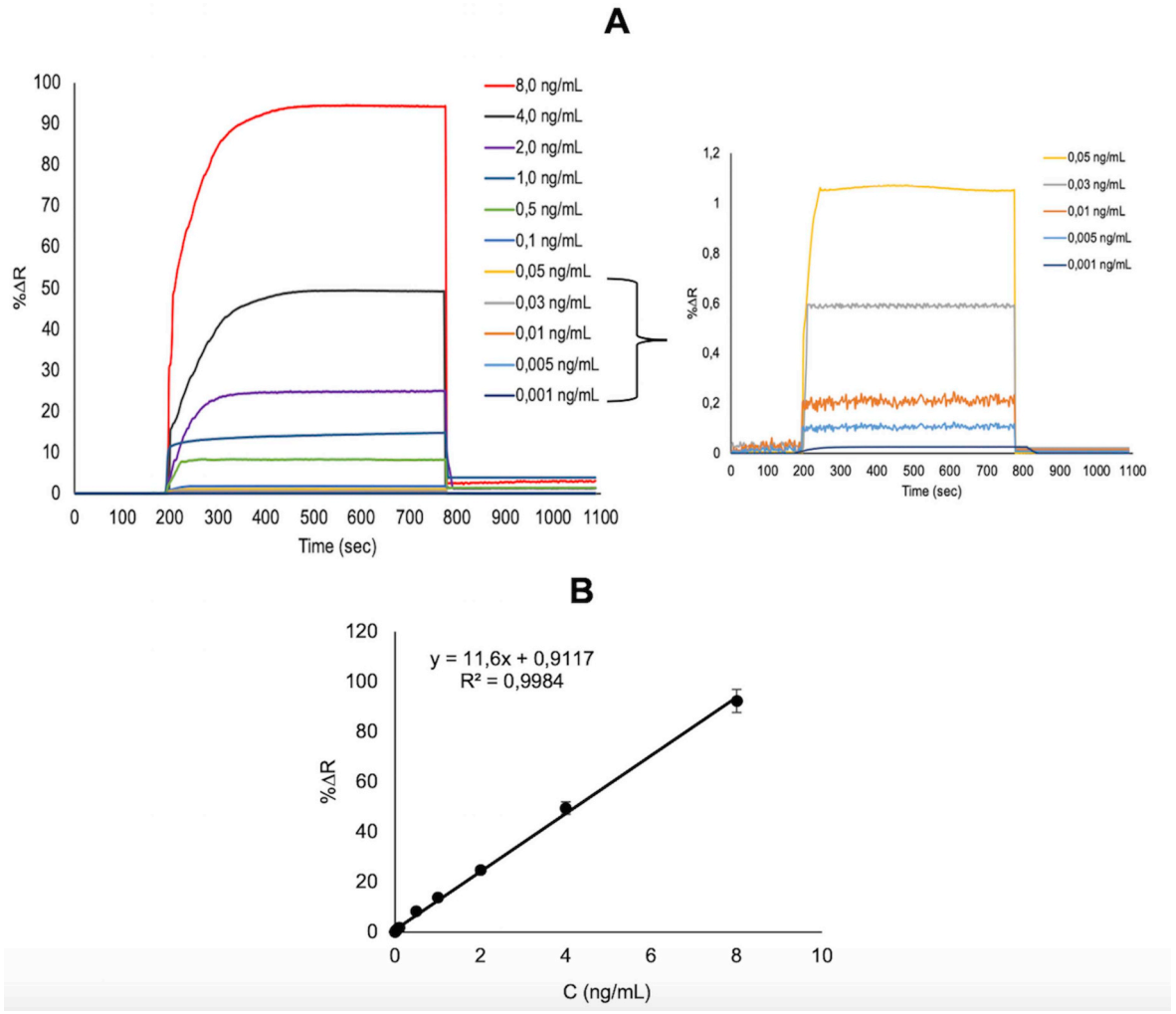


**Fig. 4. A.** Response of SPR biosensor for cardiac troponin I (cTnI) (pH: 7.4 phosphate buffer, concentrations range: 0.001–8.0 ng/mL, flow rate: 2.0 mL/min, $V_T$: 5 mL, T: 25 °C), **B.** Calibration curve of different cardiac troponin I (cTnI) concentration.

**Table 1**
Comparison of the most sensitive platform technologies for real time detection of cardiac troponin I (cTnI).

| Technology | Linear Range | Clinical Sample | LOD | Ref |
|---|---|---|---|---|
| High Electron Mobility Transistor Biosensors | 0.006–148 ng/mL | Clinical serum | – | [11] |
| Immune probe-assisted SPR biosensor | – | Serum | 3.75 ng/mL | [36] |
| Electrochemical Aptasensor | 1 – 10 000 pM | Human serum | 1 .0 pM | [37] |
| Electrochemical aptasensor | 0.05–500 ng/mL | Blood samples | 8.0 pg/mL | [38] |
| Colorimetric sensing | – | Serum | 0.2 ng/mL | [39] |
| Immunoassay | – | Human serum | 0.032 ng/mL | [40] |
| Nanobiosensor | – | Human serum | 1 pg/mL | [41] |
| Immunosensor | 5.0 pg/mL-20 ng/mL | Human serum | 1.756 pg/mL | [42] |
| Sandwich-type electrochemical immunosensor | 0.5 pg/mL-10 ng/mL | Human serum | 0.17 pg/mL | [43] |
| Electrochemical immunosensor | 10–100 pg/mL | Serum | 1.9 pg/mL | [44] |

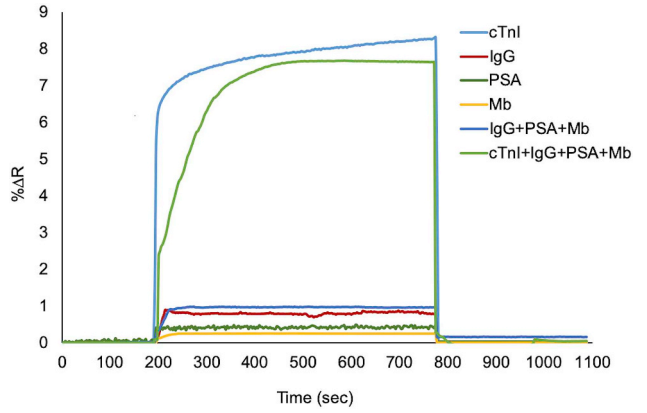


**Fig. 5.** Measured response to cardiac troponin (cTnI) and other non-specific protein (myoglobin (Mb), immunoglobulin G (IgG) and prostate specific antigen (PSA)) with the same concentration of 0.5 ng/mL in PBS buffer, flow rate: 2.0 mL/min, ($V_{solution}$: 5 mL, T: 25 °C).

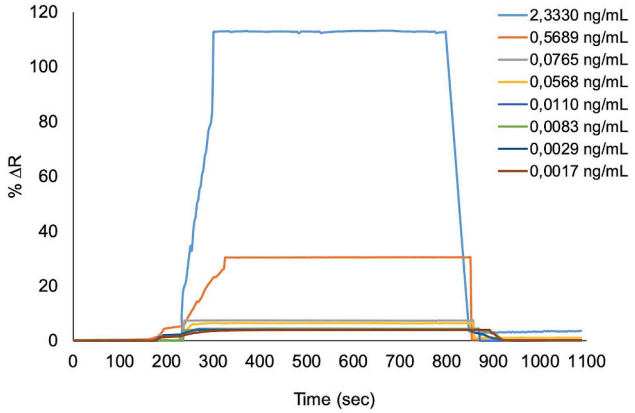


**Fig. 6.** Sensorgram for real-time cardiac troponin (cTnI) detection in 1/4 diluted patient plasma samples with pH: 7.4 phosphate buffer, flow rate: 2.0 mL/min, ($V_{solution}$: 5 mL, T: 25 °C).

(Mb + IgG + PSA) solution in competitive manner. The concentration of cTnI, Mb, IgG and PSA were kept constant as 0.5 ng/mL. Fig. 5 showed the responses of SPR biosensor for Mb, IgG and PSA were lower than cTnI.

### 3.3. Clinical analysis

Determination of the low concentrations of cardiac biomarkers in patient serum samples is very important for early diagnosis of cardiac troponin I (cTnI). The level of cTnI concentration is around 0.001 ng/mL in normal patients. The level of cTnI concentration is around 0.1 ng/mL in acute heart failure [10]. Cardiac troponin I (cTnI) detection was performed from patients serum samples. Patients serum samples were diluted in the ratio of 1/4 (pH 7.4 phosphate buffer). The sensorgrams were real-time monitored on the SPR system and were shown in Fig. 6 and Table 2. From the obtained kinetic data, troponin concentrations were determined from serum samples of patients. The recovery (%) was calculated to determine the accuracy and reliability of SPR biosensor and ELISA (Table 2). Patient serum samples encoded with numbers 6, 7 and 8 are in the low-risk group. Especially, the patient serum sample encoded with number 8 is a healthier individual. Patient serum samples encoded with numbers 3, 4 and 5 are in the middle risk group. Patients serum samples coded with numbers 1 and 2 are in the high-risk group. It was observed that the obtained ELISA results were consistent with the results of SPR biosensors. The results showed that SPR biosensor provide an accurate, sensitive, quantitative assay and reliability for measurement of cardiac troponin I (cTnI).

### 3.4. Reproducibility and stability

In the reusability study, cardiac troponin I (cTnI) solutions were prepared in pH 7.4 phosphate buffer solutions at 0.5 ng/mL cardiac troponin I (cTnI) concentration. The equilibration-adsorption-regeneration cycles were applied with the use 0.5 ng/mL concentration of cardiac troponin I (cTnI) solutions under the same conditions. As can be seen from the Fig. 7, SPR biosensors have displayed reproducible resonance change (%ΔR) during the five cycles. After five repeated cycles, SPR biosensors were showed SPR that there was no any decrease in binding capacity during the five cycles. It showed that SPR biosensors were stable under long-term storage conditions. In addition, SPR biosensors were tested at different times (1, 3, 9 and 27 months) to show storage stability. After 3 months of storage stability of the SPR biosensors, 84.16% for cardiac troponin I of the initial activity of the SPR biosensors remained. The equilibration-adsorption-regeneration shifts in % change in reflectivity (ΔR) was shown in Fig. 7A. The experiments which were statistically evaluated were repeated for five times in an intraday study. Precision values showing reproducibility were calculated as standard deviation (SD) and relative standard deviation (RSD

**Table 2**
The recoveries of cardiac troponin (cTnI) in patient plasma samples (n = 3).

| Patient Number | Found cTnI (ng/mL) | | Recovery (%) |
|---|---|---|---|
| | ELISA | SPR | |
| 1 | 2.360 | 2.333 | 98.86 |
| 2 | 0.5728 | 0.5689 | 99.32 |
| 3 | 0.0775 | 0.0765 | 98.71 |
| 4 | 0.0576 | 0.0568 | 98.61 |
| 5 | 0.0113 | 0.0116 | 102.65 |
| 6 | 0.0086 | 0.0083 | 96.51 |
| 7 | 0.0030 | 0.0029 | 96.67 |
| 8 | 0.0017 | 0.0017 | 100.00 |

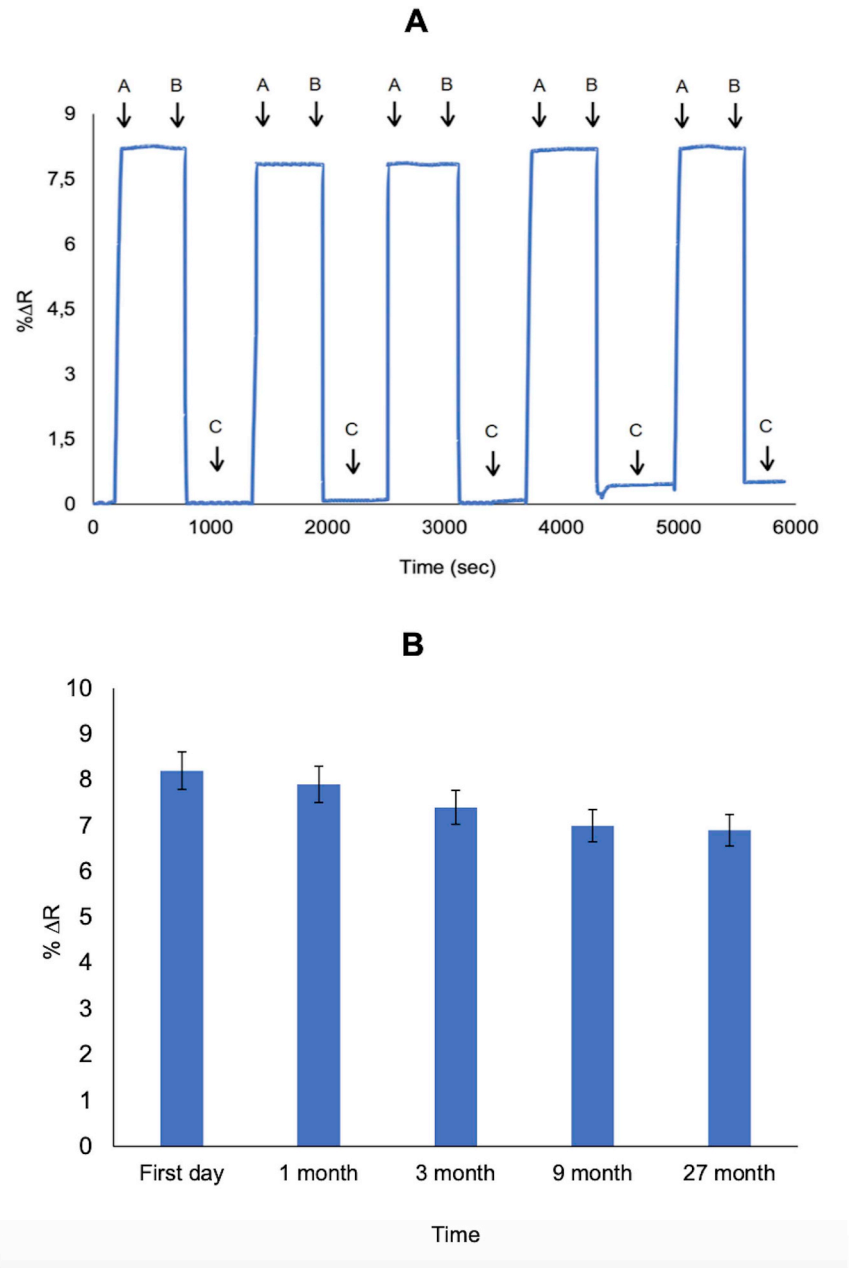


**Fig. 7.** Reproducibility **(A)** and long-term stability **(B)** of SPR biosensors ($C_{(cTnI)}$: 0.5 ng/mL, flow rate: 2.0 mL/min, $V_{solution}$: 5 mL, T: 25 °C in all measurements, A: equilibration, B: adsorption, C: regeneration for reproducibility image).

%). The standard deviation (SD) and relative standard deviation (RSD %) were 0.109 and 1.354, respectively. The obtained RSD% value for intraday accuracy were ≤2.00%. The RSD value showed that the proposed method has a good sensitivity [45].

## 4. Conclusions

In this study, we developed a new methods to evaluate binding of cardiac troponin I (cTnI). The real time detection of cardiac troponin I (cTnI) is challenging and it is crucial for clinical studies. In this study, we were prepared anti-cardiac troponin I monoclonal antibody immobilized SPR biosensors for detection of cardiac troponin I (cTnI). SPR biosensor has been successfully used for the real-time detection of cardiac troponin I (cTnI) from aqueous solution and patient serum samples by SPR method. SPR biosensor was showed high sensitivity, real-time monitoring, low detection concentration and low volume sample against other analytical methods. The presented system can be deployed as a cell surfaceome array in cardio-specific biomarker discovery and can integrated with immunoassay, clinical analysis and diagnosis of disease, healthcare monitoring, immunostaining and bioinformatics. Further, the data can used more detailed characterization of cardiac troponin I (cTnI) on SPR chip surface. In this study, we present new avenues on sensing modalities by significantly reducing the cost, assay time (13.30 min) and providing an easy-to-use for real-time monitoring the kinetic binding profiles of the molecule.

## Credit author statement

D.Ç. prepared SPR chip surface. D.Ç and N.B. conducted the experiments and data analyses. A.D and S.G. supervised the data analyses and interpretation of the results. N.B. and D.Ç. wrote the manuscript with input from all authors. All authors provided critical feedback and

helped shape the research, analysis, and manuscript.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2020.121259.

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